The University Of Sheffield.

Improving Tissue Engineered Repair Materials Used in the Treatment of Pelvic Floor Diseases

By:

Christopher J. Hillary

A thesis submitted for the degree of Doctor of Philosophy

The University of Sheffield

Faculty of Materials Science and Engineering

January 2017

For my wonderfully supportive and patient wife, Sarah

Table of Contents

TABLE OF CONTENTS 3

ABSTRACT 10

AIMS AND OBJECTIVES 12

2.1 AIMS AND OBJECTIVES 12

2.2 HYPOTHESES 13

PUBLICATIONS, PRESENTATIONS & GRANTS AWARDED 14

PUBLICATIONS 14

Research manuscripts 14

Review articles 14

PRESENTATIONS 15

GRANTS AWARDED 16

ACKNOWLEDGEMENTS 17

ABBREVIATIONS 18

LIST OF FIGURES 22

LIST OF TABLES 25

CHAPTER I: INTRODUCTION 26

1.1 STRESS URINARY INCONTINENCE 27

1.1.1 Definitions 27

1.1.2 Prevalence 28

1.1.3 Aetiology 29

1.1.3.1 Age 29

1.1.3.2 Parity and vaginal delivery 30

1.1.3.3 Obesity 31

1.1.3.4 Family history 32

1.1.4 Health Economics 32

1.1.5 Quality of Life (QoL) 32

1.2 PELVIC ORGAN PROLAPSE 33

1.2.1 Definitions 33
1.2.2 Prolapse classification 34
1.2.3 Prevalence 36
1.2.4 Aetiology 36
1.2.4.1 Pelvic surgery 36
1.2.4.2 Parity and vaginal delivery 37
1.2.4.3 Obesity 39
1.2.4.4 Connective tissue diseases 39
1.2.4.5 Family history 39
1.2.5 Health economics 39
1.2.6 Quality of life (QoL) 40
1.3 PELVIC FLOOR ANATOMY 41
1.3.1 Levator ani muscle complex 41
1.3.2 The urogenital diaphragm 42
1.3.3 The perineal body 42
1.3.4 Parietal and endopelvic fascia 42
1.3.5 Ligaments 43
1.3.6 Anterior vaginal wall 44
1.3.7 Posterior vaginal wall 44
1.3.8 Levels of support 44
1.3.9 Female continence structures 45
1.4 PELVIC FLOOR DYSFUNCTION 48
1.4.1 Mechanism for the development of SUI 48
1.4.1.1 Urethral hypermobility 48
1.4.1.2 Intrinsic sphincter deficiency 50
1.4.2 Mechanism for the development of POP 51
1.5 PATHOGENESIS OF PELVIC FLOOR DYSFUNCTION 55
1.5.1 The vaginal wall 55
1.5.2 Musculo-ligamentous structural changes 56
1.5.3 Extracellular matrix proteins 57
1.5.4 Clinical significance 61
1.5.4.1 Collagen content in the diseased state 62
1.5.4.2 Elastin content in the diseased state 63
1.5.5 Function of fibroblasts in the ECM 66
1.5.6 Effect of oestradiol on ECM component production 66
1.6 BIOMECHANICS OF THE PELVIC FLOOR CONNECTIVE TISSUE 68
1.6.1 Mechanical testing 68

1.6.1.1 Tensile testing 69
1.6.1.2 Problems with tensile testing 71
1.6.2 Biomechanical properties of connective tissues 71
1.6.3 Review of the biomechanical properties of pelvic floor tissues 72
1.7 TREATMENTS FOR POP/SUI 75
1.7.1 Conservative treatment of pelvic floor disorders 76
1.7.2 Mechanical devices for prolapse 77
1.7.3 Medical treatment of pelvic floor disorders 77
1.7.4 Biomaterials for SUI and POP 78
1.7.4.1 Injectable materials for SUI 79
1.7.4.1.1 Outcomes 79
1.7.4.2 Synthetic mesh for SUI/POP 82
1.7.4.3 Biological grafts used for SUI/POP 84
1.7.5 Surgical outcomes in SUI/POP 86
1.7.5.1 SUI surgery – Native tissue repair (NTR) 86
1.7.5.1.1 The Marshall-Marchetti-Krantz (MMK) procedure 86
1.7.5.1.2 Burch colposuspension (BC) 87
1.7.5.1.3 Paravaginal repair 87
1.7.5.1.4 Vagino-obturator shelf repair (VOS repair) 88
1.7.5.1.5 Laparoscopic retropubic suspension 88
1.7.5.2 SUI surgery – biomaterial sling/tape procedures 89
1.7.5.2.1 Pubovaginal sling (PVS) 89
1.7.5.2.2 Mid-urethral tape (MUT) 91
1.7.5.3 Comparative data – Bulking agents versus surgery 94
1.7.5.4 Comparative data – sling/tape procedures versus NTR 94
1.7.5.5 Comparative data – biomaterials used during sling/tape procedures 95
1.7.5.6 Surgery for POP – Apical compartment 97
1.7.5.6.1 Native tissue repair 97
1.7.5.6.2 Biomaterial repair 97
1.7.5.7 Surgery for POP – Anterior compartment 98
1.7.5.7.1 Native tissue repair 99
1.7.5.7.2 Biomaterial repair 99
1.7.5.8 Surgery for POP – Posterior compartment 100
1.7.5.8.1 Native tissue repair 101
1.7.5.8.2 Biomaterial repair 101
1.7.5.9 POP surgery - Conclusions 102
1.8 COMPLICATIONS OF BIOMATERIALS THAT ARE USED IN SUI/POP 104

1.8.1 Defining biomaterial complications 105
1.8.2 Mesh complications 108
1.8.2.1 Host response to implantation 108
1.8.2.1.1 Mesh structure 109
1.8.2.1.2 Biomechanics 109
1.8.2.1.3 Material degradation 109
1.8.2.2 Clinical complications - mesh toxicity 110
1.8.2.3 Clinical complications - infection and chronic pain 110
1.8.2.4 Clinical complications - voiding dysfunction 111
1.8.3 Biomaterial complications 111
1.8.3.1 Tissue processing 112
1.8.3.2 Integration and degradation 112
1.8.4 The ideal material 113
1.9 REGENERATIVE MEDICINE AND TISSUE ENGINEERING TECHNIQUES FOR PELVIC FLOOR RECONSTRUCTION 115
1.9.1 Regenerative medicine approaches 115
1.9.1.1 Bone marrow derived stem cells 116
1.9.1.2 Muscle derived stem cells 116
1.9.1.3 Adipose derived stem cells 117
1.9.2 Tissue engineering approaches 119
1.9.2.1 Cells 119
1.9.2.2 Scaffolds 120
1.10 BACKGROUND TO THE PROJECT 123
1.10.1 Formation of matrices – Electrospinning 123
1.10.2 Chemo-stimulation 124
1.10.2.1 Antimicrobials and anti-inflammatories 125
1.10.2.2 Drug releasing scaffolds that improve vasculogenesis 126
1.10.2.3 Oestradiol 126
1.10.2.4 Vitamin C 127
1.11 THE IMPORTANCE OF BIOMECHANICAL PROPERTIES 128
1.12 PREVIOUS WORK AT OUR INSTITUTION 129

CHAPTER II: MATERIALS AND METHODS 130

2.1 SCAFFOLDS AND CELLS 131
2.1.1 Polymer preparation 131
2.1.2 Electrospinning 131

2.1.2.1 Basic electrospinning of random fibres 131
2.1.2.2 Electrospinning two separate polymers (co-polymers) 132
2.1.2.3 Electrospinning layered polymer scaffolds 132
2.1.3 Isolation and culture of adipose derived stem cells 134
2.2 SAMPLE PREPARATION AND CELL SEEDING 135
2.3 DRUG RELEASING SCAFFOLDS 136
2.3.1 Drug releasing scaffolds 136
2.3.2 Measurement of oestradiol release 136
2.3.3 Calculation of viable oestradiol dose 137
2.3.4 Cell differentiation assays in 2-D 137
2.4 MEASUREMENT OF CELL RESPONSE 139
2.4.1 Metabolic activity 139
2.4.2 Staining for total collagen production 139
2.4.3 Cell attachment and matrix production – immunofluorescence 140
2.4.4 Examination of cell penetration into scaffolds 140
2.4.5 Mechanical testing under static and dynamic conditions 141
2.4.6 Scanning electron microscopy 143
2.4.7 Statistics 143
2.5 IN VIVO EXAMINATION OF SCAFFOLDS 144
2.5.1 Polymers 144
2.5.2 Scaffold production 144
2.5.3 Animals 145
2.5.4 Implantation 146
2.5.5 Animal Sacrifice 148
2.5.6 Biomechanical Testing 148
2.5.7 Histology 150
2.5.8 Statistics 151

CHAPTER III: THE PRODUCTION AND CHARACTERIZATION OF ELECTROSPUN POLYMER SCAFFOLDS 153
3.1 CHAPTER INTRODUCTION 154
3.2 SCAFFOLD MORPHOLOGY 155
3.2.1 Fibre morphology 156
3.2.2 Fibre diameter 156
3.2.3 Pore diameter 156
3.2.4 Section discussion 160
3.3 MECHANICAL PROPERTIES OF SCAFFOLDS 162

3.3.1 Tensile test – dry materials 162
3.3.2 Young's modulus – dry materials 162
3.3.3 Ultimate tensile strength – dry materials 162
3.3.4 Plastic deformation 163
3.3.5 Effect of dynamic loading under bioreactor conditions 163
3.3.6 Section discussion 169
3.4 CELLULAR RESPONSE TO SCAFFOLDS 171
3.4.1 Cell metabolic activity 171
3.4.2 Total collagen production 171
3.4.3 Matrix deposition on scaffolds 171
3.4.4 Mechanical properties 172
3.4.5 Investigation of cell penetration using fluorescence microscopy 172
3.4.6 Section Discussion 180
3.5 CHAPTER DISCUSSION 182

CHAPTER IV: BIOMIMETIC SCAFFOLDS 185

4.1 CHAPTER INTRODUCTION 186
4.2 STRUCTURE AND FUNCTION 187
4.2.1 Fibre morphology 187
4.2.2 Fibre size and porosity 187
4.2.3 Oestradiol release 188
4.2.4 Section discussion 192
4.3 CELLULAR AND MECHANICAL OUTCOMES 194
4.3.1 Assessment of cellular differentiation in response to oestradiol 194
4.3.2 Cell metabolic activity 194
4.3.3 Total collagen production 195
4.3.4 Matrix component production 195
4.3.5 Mechanical properties 195
4.3.6 Section discussion 200
4.4 CHAPTER DISCUSSION 201

CHAPTER V: IN VIVO ASSESSMENT OF REPAIR MATERIALS 204

5.1 CHAPTER INTRODUCTION 205
5.1.1 Material explantation 206
5.2 MECHANICAL OUTCOMES 209
5.2.1 Mechanical properties of repair materials 209

5.2.1.1 Young's modulus 209
5.2.1.2 Ultimate tensile strength 209
5.2.2 Mechanical properties of repair site 210
5.2.2.1 Youngs modulus 210
5.2.2.2 Ultimate tensile strength 210
5.2.3 Section discussion 213
5.3 IMMUNOHISTOLOGICAL OUTCOMES 215
5.3.1 Sample integration 215
5.3.2 Collagen III production and macrophage infiltration 216
5.3.3 New blood vessel formation and lymphocyte infiltration 216
5.3.4 Macrophage phenotype 216
5.3.5 Section discussion 223
5.4 CHAPTER DISCUSSION 225

CHAPTER VI: FINAL DISCUSSION AND CONCLUSIONS 228

6.1 DISCUSSION 229
6.2 CONCLUSIONS 233
6.3 FUTURE DIRECTIONS 234

APPENDICES 235

APPENDIX 1 236
APPENDIX 2 237
APPENDIX 3 238
APPENDIX 4 239
APPENDIX 5 240
APPENDIX 7 241
APPENDIX 7 242

REFERENCES 243

Abstract

Introduction: Tissue engineering and regenerative medicine techniques may offer improved outcomes for patients suffering from pelvic organ prolapse (POP) and stress urinary incontinence (SUI) compared to polypropylene mesh, which is associated with significant complications. Complications with polypropylene are purported to occur due to an excessive immunological reaction and a mismatch of mechanical properties.

Aim: To produce scaffolds with improved biomechanical properties using electrospun degradable and non-degradable scaffolds and to produce oestradiol-releasing scaffolds, which will to improve tissue integration.

Methods: Electrospinning was used to produce poly-L-lactic acid and polyurethane scaffolds. Oestradiol-releasing poly-L-lactic acid scaffolds were produced by dissolving the drug in the polymer solution prior to electrospinning. Mechanical properties of these materials were measured using a BOSE tensiometer both before and after cyclical distension. Cell metabolic activity and total collagen production were measured using AlamarBlue assay and Sirius red assay respectively. Oestradiol was incorporated into scaffolds and its release measured fluorimetrically over a 5 month period. The effects of oestradiol on cells in culture were measured (AlamarBlue and Sirius red), and differentiation assays were performed using specific induction media. Specific extracellular component production was assessed using immunohistochemistry. Cell penetration was assessed using confocal microscopy techniques. Poly-L-lactic acid and polyurethane scaffolds were implanted into abdominal wall defect rabbit models over a 3 month period, with histologial, mechanical and immunological outcomes measured.

Results: Poly-L-lactic acid scaffolds demonstrated a greater ability of cells to penetrate and showed greater outcomes for cell viability assays and collagen production when stem cells were cultured on them. Meanwhile, polyurethanes demonstrated significantly greater elastic properties, that remained unchanged following periods of cyclical distension. Commercially available

polypropylene mesh became plastically deformed following even short periods of cyclical distension to 25% displacement of the original length of the material. Oestradiol-releasing scaffolds were produced, that released the drug over a 5-month period in a dose dependent fashion. Scaffolds that release oestradiol demonstrated a significantly greater total collagen production, which resulted in a stronger biomaterial. Following implantation in animal models, polypropylene mesh was associated with tissue exposure. Poly-L-lactic acid scaffolds became well integrated into the host and became replaced with host cells. Both poly-L-lactic acid and polyurethane scaffolds demonstrated a predominantly M2 macrophage response, while polypropylene mesh showed an M1 macrophage phenotype. The biomechanical properties of tissues that were repaired by either of poly-L-lactic acid, polyurethane, or polypropylene were stronger than tissues that were not reinforced with repair materials.

Conclusions: Poly-L-lactic acid scaffolds demonstrate excellent tissue integration and regeneration, while polyurethanes offer mechanical properties that are more closely related to healthy fascia. Oestradiol-releasing scaffolds can support greater proliferation and collagen production of cultured cells, which can be associated with better integration into the host following implantation. Meanwhile, polypropylene mesh exhibits plastic deformation following cyclical distension and is associated with an excessive immunological reaction, which could explain the complications that are observed with this material in patients undergoing mesh surgery.

Aims and objectives

The overall aim is to undertake research into developing materials designed for supporting tissues in the pelvic floor seeking to produce materials which are a better biomechanical match for the patients tissues than the PPL material in current use and to develop approaches to improve materials, which integrate readily into the patient. The specific objectives are as follows:

3 To assess the current treatments for stress urinary incontinence and pelvic organ prolapse and to critically investigate why these fail.

4 To improve upon the mechanical properties of current materials by investigating a range of novel synthetic materials.

4.1 Optimise electrospinning protocols.

4.2 Assess morphology of these scaffolds using scanning electron microscopy.

4.3 Examine cell viability and collagen production.

4.4 Measure mechanical properties.

4.5 Investigate the most promising of these materials *in vivo*.

5 To examine methods to produce scaffolds that release oestradiol.

5.1 Identify methods to incorporate oestradiol into scaffolds.

5.2 Measure the release of oestradiol.

5.3 Perform cell viability assays to determine appropriate dosages.

6 To investigate the response to candidate materials following implantation in animal models.

6.1 Assess mechanical response to implantation.

6.2 Identify immunohistological outcomes of materials following implantation.

Hypotheses

1. The mechanical properties of tissue engineered prostheses can be made to more closely resemble those of healthy paravaginal tissues.
2. Scaffolds can be produced to release certain drugs (oestradiol) that will increase local collagen production and improve vasculogenesis.
3. Cell penetration into materials can be improved through modifying the fabrication of materials.
4. Novel scaffolds can demonstrate a greater constructive remodeling process than currently used polypropylene mesh.

Publications, Presentations & Grants awarded

Publications

Research manuscripts

- **Oestradiol-releasing biodegradable mesh stimulates collagen production and angiogenesis: An approach to improving biomaterial integration in pelvic floor repair.** Mangir N, Hillary CJ, Chapple C, MacNeil S. *European Urology Focus*. 2017
- **Developing repair materials for stress urinary incontinence to withstand dynamic distension.** Hillary CJ, Roman S, Bullock AJ, Green NH, Chapple C, MacNeil S. *PLOS One.* 2016
- **Evaluating alternative materials for the treatment of stress urinary incontinence and pelvic organ prolapse –a comparison of the *in vivo* response to meshes of polypropylene, polyvinylidene fluoride, poly-lactic acid and polyurethane implanted in rabbits for 3 months.** Roman S, Urbankova I, Callewaert G, Lesage F, Hillary CJ, Osman N, Chapple C, Deprest J, MacNeil S. *Journal of Urology*. 2016

Review articles

- **Tissue engineered buccal mucosa for urethroplasty: Progress and future directions.** Osman NI, Hillary C, Bullock AJ, MacNeil S, Chapple C. *Advanced drug delivery reviews*. 2014.
- **Recent developments in technology for the assessment and management of incontinence**. Hillary CJ, Slovak M, McCarthy A, Hashim H, Chapple C. *Journal of Medical Engineering and Technology*. 2015.
- **Application of tissue engineering to pelvic organ prolapse and stress urinary incontinence.** Chapple C, Osman N, Mangera A, Hillary CJ, Roman S, Bullock A, MacNeil S. *LUTS*. 2015

Presentations

- **Engineered drug eluting scaffolds for the treatment of pelvic floor disorders.** Hillary C, Bullock A, MacNeil S, Chapple C. Oral presentation at *BiTEG*, Leeds. 2013.
- **Tissue engineered oestradiol releasing slings for the treatment of stress urinary incontinence.** Hillary C, Bullock AJ, Osman N, MacNeil S, Chapple C,. Poster presentation at *European Association of Urology* annual meeting, Stokholm. 2014.
- **The role of fabricated oestradiol releasing synthetic scaffolds for the treatment of stress urinary incontinence (SUI) and pelvic organ prolapse (POP)**. Hillary C, Bullock AJ, Chapple C, MacNeil S. Poster presentation at the *British Association of Urological Surgeons* Section of Academic Urology annual meeting. Royal College of Surgeons of England. December 2014.
- **Response of scaffold materials designed for treatment of stress urinary incontinence (SUI) and pelvic organ prolapse (POP) to distension.** Hillary C, Roman S, Bullock AJ, Chapple C, MacNeil S. Extend oral presentation at the *British Association of Urological Surgeons* Section of Academic Urology annual meeting. Royal College of Surgeons of England. December 2014.
- **The *in vitro* response to fabricated oestradiol releasing scaffolds designed for the treatment of pelvic floor disorders.** Hillary C, Bullock AJ, Chapple C, MacNeil S. Oral presentation at *European Association of Urology* annual meeting, Madrid
- **Composite Materials for Pelvic Floor Repair Which Cope with Dynamic Distension and Support Cell Integration**. MacNeil, S, Hillary, CJ, Roman S, Bullock AJ, Chapple CR. Oral presentation at *TERMIS*, Boston. 2015.
- **Estradiol Releasing Scaffolds for the Treatment of Pelvic Floor Disorders.** MacNeil, S, Hillary, CJ, Roman S, Bullock AJ, Chapple CR. Oral presentation at *TERMIS*, Boston. 2015.
- **Response of Scaffold Materials Designed for Treatment of Stress Urinary Incontinence (SUI) and Pelvic Organ Prolapse (POP) to Distension.** Hillary CJ,

Roman S, Bullock AJ, Chapple CR, MacNeil S. Oral presentation at the *International Continence Society* annual meeting, Montreal 2015.

- **The in Vivo Response to Implantable Engineered Repair Materials Designed for Pelvic Floor Reconstruction.** Roman S, Hillary CJ, Urbankova I, Callewaert G, Lesage F, Deprest J, Chapple C, ManNeil S. Oral presentation at the *International Continence Society* annual meeting, Montreal 2015.

Grants awarded

- **The Urology Foundation Scholarship 2013 (£35,000).** Developing materials for the treatment of SUI and POP, which cope well with distension. June 2013 to June 2014.
- **The Urology Foundation Scholarship 2014 (£22,520).** Developing fascia-mimetic estradiol releasing scaffold materials for the treatment of stress urinary incontinence and pelvic organ prolapse. July 2014 to Aug 2015.
- **The Urology Foundation Scholarship 2015 (£28,426).** Bioactive mid-urethral slings for stress urinary incontinence. Aug 2015 to July 2016.

Acknowledgements

In undertaking this PhD degree, I am extremely grateful to several key individuals, who have supported me, provided motivation, taught me, and pushed me to become a better researcher.

Firstly, I owe huge thanks to Professor Sheila MacNeil and Professor Christopher Chapple, without whom this project would not exist and whose invaluable guidance brought much needed clarity and encouraged me to persevere when experiments did not quite go to plan. Both of these mentors provided me with some fantastic opportunities during this project, for which I will be eternally grateful. I am also sincerely thankful to Karen Heard and Jen Tidman for their time, patience and support with all things technical.

I owe a huge debt of gratitude to the many new friends I have made at the University – Dr Sabiniano Roman, Dr Anthony Bullock, Dr Naside Mangir and Mr Nadir Osman. Their technical support, teaching and collaboration made my research experiences so enjoyable and I am sure that this will continue.

I would like to thank Mr Derek Rosario, Mr David Yates, Mr Richard Inman, Professor Jim Catto and Mr Anthony Browning, along with several other of my clinical supervisors, whose patience (and occasional berating) allowed me to complete this thesis.

I am grateful to my parents, Debbie and Alan for their continued understanding and support and to all of my family. This work would not be possible without the generosity of The Urology Foundation.

Finally, I dedicate this culmination of 4 years work to my loving wife, Sarah. Your kindness and support has made this all possible.

Christopher James Hillary

Sheffield, UK

2017

Abbreviations

ADSC	Adipose derived stem cells
ASC	Abdominal sacrocolpopexy
ATFP	Arcus tendineus fascia pelvis
ATRV	Arcus tendineus rectovaginalis
ATLA	Arcus tendineus levator ani
BC	Burch colposuspension
BMDSC	Bone marrow derived stem cell
BMI	Body mass index
BOO	Bladder outflow obstruction
BSA	Bovine serum antigen
CI	Confidence interval
CISC	Clean intermittent self catheterisation
COPD	Chronic obstructive pulmonary disease
DAPI	4′,6-diamidino-2-phenylindole dihydrochloride
DCM	Dichloromethane
DMEM	Dulbecco's modified Eagles medium
DMF	Dimethylformide
DNA	Deoxyribonucleic acid
DO	Detrusor overactivity
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
EToH	Ethanol
FBR	Foreign body reaction
FCS	Foetal calf serum
FDA	Food and drug administration
FITC	Fluorescein isothiocyanate
GAG	Glycosaminoglycans
GH	Genital hiatus
HMDI	Hexamethylenediisocyanate
HRQoL	Health related quality of life

ICI	International consultation on incontinence
ICIQ	International consultation on incontinence modular questionnaire
ICS	International continence society
ICTP	Carboxyterminal telopeptide
IMS	Industrial methylated spirit
ISD	Instrinsic sphincter deficiency
IUGA	International urogynaecology association
LUTS	Lower urinary tract symptoms
MAUDE	Manufacturer and user facility device experience
MDSC	Muscle derived stem cell
MHRA	Medicines and healthcare regulatory agency
MMK	Marshall-marchetti-krantz procedure
MMP	Metalloproteinases
MPa	Megapascals
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cell
MUCP	Maximal urethral closure pressure
MUI	Mixed urinary incontinence
MUT	Midurethral tape
N	Newtons
NaOH	Sodium hydroxide
NIH	National institute of health
NHS	National health service
NTR	Native tissue repair
OAB	Overactive bladder
OF	Oral fibroblast
OR	Odds ratio
Pa	Pascals
PB	Perineal body
PBS	Phosphate buffered saline
PCL	Polycaprelactone
PET	Polyethylene terepthalate
PFRM	Pelvic floor repair material

PFS	Pressure flow study
PGA	Polyglycolic acid
PHBV	Polyhydroxybutyrate-co-hydroxyvalerate
PICP	Procollagen type I carboxyterminal propeptide
PLA	Poly-L-lactic acid
PLGA	Polylactide-co-glycolide
PMC	Pontine micturition centre
POP	Pelvic organ prolapse
POP-Q	Pelvic organ prolapse quantification system
PPL	Polypropylene
PTFE	Polytetrafluoroethane
PVA	Polyvinylacetate
PVS	Pubovaginal sling
PU	Polyurethane
RCT	Randomized controlled trial
RR	Relative risk
SC	Sacrocolpopexy
SEM	Standard error of mean
SEM	Scanning electron microscopy
SHG	Second harmonic generation
SIS	Small intestine submucosa
SLRPs	Small leucine-rich repeat proteoglycans
SSLS	Sacrospinous ligament suspension
SVF	Stromal vascular fraction
SUI	Stress urinary incontinence
TGF-β1	Transforming growth factor β1
THF	Tetrahydrofuran
TIMPS	Tissue-derived inhibitors of MMPs
TOT	Transobturator tape
TVL	Total vaginal length
TVT	Transvaginal tape
USS	Ultrasound
USI	Urodynamic stress urinary incontinence

USLS	Uterosacral ligament suspension
UTS	Ultimate tensile strength
UUI	Urgency urinary incontinence
VEGF	Vascular endothelial growth factori
VLPP	Valsalva leak point pressure
VOS	Vagino-obturator shelf procedure
QoL	Quality of life
TIMP	Tissue derived inhibitor of MMPs
YM	Young's modulus
Z1	Polyurethane z1a1
Z3	Polyurethane z3a1
2D	Two dimension
3D	Three dimension

List of figures

Figure 1.1.1. Prevalence of SUI, UUI, and MUI by age 30

Figure 1.1.2. Grouped odds ratios of SUI, UUI, and MUI according to BMI 31

Figure 1.2.1. Pelvic Organ Prolapse Quantification System (POP-Q) 35

Figure 1.2.2. Rate of POP surgery by mode and timing of delivery 38

Figure 1.3.1. Gross anatomy of the female pelvic floor musculo-ligamentous attachments .. 43

Figure 1.3.2. Continence structures of the female pelvic floor 47

Figure 1.4.1. Pressure transmission theory of stress urinary incontinence 49

Figure 1.4.2. Hammock theory of stress urinary incontinence 50

Figure 1.4.3. Mechanisms for the development of pelvic floor dysfunction..... 53

Figure 1.4.4. Paramore's theory of pelvic floor dysfunction 54

Figure 1.5.1. Histology of the vaginal wall 56

Figure 1.5.2. Diagrammatic representation of collagen synthesis.....60

Figure 1.5.3. Representative immunohistochemistry images of uterosacral ligament specimens of pre and post-menopausal women with and without POP 65

Figure 1.6.1. Representation of stress-strain curve for connective tissues that behave in a non-linear mechanical fashion 70

Figure 1.6.2. Viscoelasticity of tissues 72

Figure 1.7.1. Technique for the injection of urethral bulking agents..... 81

Figure 1.7.2. Microscopic appearances of surgical mesh 83

Figure 1.7.3. The Burch colposuspension 87

Figure 1.7.4. Donor site options for fascial strips – rectus fascia and fascia lata of the thigh .. 90

Figure 1.7.5. Autologous fascia sling-on-a-string procedure 91

Figure 1.7.6. The TVT – tension-free vaginal tape procedure 93

Figure 1.10. Electrospinning apparatus 124

Figure 2.1.1. The formation of random electrospun fibres 133

Figure 2.1.2. The formation of random-aligned co-polymers 133

Figure 2.4.1. Cell metabolic activity of cultured cells seeded on scaffolds in a well-plate..... 139

Figure 2.4.2. Measuring the tensile properties of tissues/materials using a tensiometer 142

Figure 2.4.3. Calculation of Young's modulus and ultimate tensile strength from a stress-strain curve 142

Figure 2.4.4. Programmable cyclical distension in a closed incubator bioreactor 143

Figure 2.5.1. Formation of random-aligned PLA co-polymers using a novel multichannel needle 145

Figure 2.5.2. Diagrammatic representation of implantation technique 147

Figure 2.5.3. Representative photographs to demonstrate implantation technique 147

Figure 2.5.4. Biomechanical testing of explanted samples 150

Figure 3.2.1. Fibre morphology as demonstrated by scanning electron microscopy..... 157

Figure 3.2.2. Fibre diameter of scaffolds 158

Figure 3.2.3. Pore diameter of scaffolds 158

Figure 3.3.1. Stress vs strain plots of 6 different materials 164

Figure 3.3.2. Mechanical properties before and after 10 cycles of deformation 165

Figure 3.3.3. Hysteresis loops of different materials over 5 cycles 166

Figure 3.3.4. Stress vs strain plots of 4 different materials 169

Figure 3.3.5. Values for mechanical properties before and after 7 days of loading.....168

Figure 3.4.1. Cell metabolic activity of candidate scaffolds.....173

Figure 3.4.2. Total collagen production of candidate scaffolds 173

Figure 3.4.3. Scanning electron microscopy images of scaffolds following 2 weeks of cell culture to demonstrate matrix deposition on scaffold surface 174

Figure 3.4.4. Stress strain curves for 5 materials using the BOSE electroforce tensiometer . 178

Figure 3.4.5. Fluorescence microscopy and scaffold fibre second harmonic generation using confocal microscopy..... 179

Figure 4.2.1. Scanning electron microscopy images of oestradiol and non-oestradiol releasing scaffolds 189

Figure 4.2.2. Calculation of fibre diameter and pore size 190

Figure 4.2.3. Release of oestradiol from PLA scaffold 191

Figure 4.3.1. Cell differentiation assays in response to oestradiol 196

Figure 4.3.2. Cell metabolic activity of ADSC 197

Figure 4.3.3. Total collagen production at day 14 197

Figure 4.3.4. Representative fluorescent microscopy images of extracellular matrix components produced by ADSC after 14 days of culture 198

Figure 4.3.5. The effect of oestradiol of the strength of the scaffolds assessed in the presence of ADSCs 199

Figure 5.1.1. Macroscopic appearance of the materials after 90 days implantation 207

Figure 5.1.2. Adhesion formation between repair material and intra-abdominal organs at 30 days 207

Figure 5.1.3. Polypropylene mesh exposure through rabbit abdominal wall 208

Figure 5.2.1. Mechanical properties of explanted repair materials 211

Figure 5.2.2. Biomechanical properties of explanted repair site 212

Figure 5.3.1. Histology of explanted samples 218

Figure 5.3.2. Immunohistochemistry staining for collagen III at day 90 219

Figure 5.3.3. Immunohistochemistry staining for macrophages at day 90 219

Figure 5.3.4. Immunohistochemistry staining for endothelial cells at day 90 220

Figure 5.3.5. Immunohistochemistry staining for T lymphocytes at day 90 220

Figure 5.3.6. Immunohistochemistry images of M1 and M2 macrophage at day 90 221

Figure 5.3.7. M2/M1 ratio for explanted materials 222

List of tables

Table 1.6. Published literature that relates to biomechanical testing of vaginal tissues.....74

Table 1.7.1. Classification of mesh by type 83

Table 1.7.2. Biological grafts used for SUI/POP surgery 85

Table 1.7.3. Bulking agents versus surgery 96

Table 1.7.4. Slings/tapes versus NTR 96

Table 1.7.5. Biomaterial repair versus PPL MUT 96

Table 1.7.6. Outcomes of biomaterials used in apical compartment surgery versus NTR and SC 103

Table 1.7.7. Outcomes of biomaterials used in anterior compartment surgery versus NTR . 103

Table 1.7.8. Outcomes of biomaterials used in posterior compartment surgery versus NTR103

Table 1.8.1. IUGA/ICS classification of complications related directly to insertion of prosthesis or grafts in female pelvic floor surgery 107

Table 1.8.2. Comparison of biomaterials used in pelvic floor reconstruction 114

Table 1.9. Summary of pre-clinical studies that investigate tissue engineering for pelvic floor repair.....122

Table 2.1. Polymer solutions used during scaffold electrospinning 131

Table 2.5. Primary antibodies and their description 152

Table 3.2. Summary of scaffold morphological properties 159

Table 3.5. Summary of scaffold properties 184

Table 6.1. Number of peritoneal adhesions occurring of 8 implants per group and time point207

Table 5.3. Dunn multiple comparisons test of M2/M1 ratio taken from figure 5.3.7 222

Chapter I: Introduction

Pelvic floor disorders in urology encompass the two conditions of stress urinary incontinence (SUI) and pelvic organ prolapse (POP). These common conditions can affect women of any age but become proportionally more prevalent with advancing age and can account for significant physical, psychological and social debility to the patient. Both SUI and POP occupy a large proportion of the NHS healthcare budget, with 1 in 10 women estimated to undergo operative management for either of these conditions during the course of their lifetime [1].

The exact mechanism behind the development of these conditions is not yet completely understood, though it is likely that pelvic floor laxity, which can result from childbirth, hormone deficiency, obesity and a genetic predisposition will play a part.

These conditions are initially treated using conservative measures, however many patients ultimately undergo surgery to achieve a cure.

1.1 Stress urinary incontinence

1.1.1 Definitions

Stress urinary incontinence (SUI) is defined by the International Continence Society (ICS) as the as 'the involuntary leakage of urine that occurs on effort or exertion' [2]. Urinary leakage is described by patients and can occur purely in the context of effort or exertion, or can occur with urinary urgency, which is described as 'the sudden compelling desire to void that is difficult to defer' [2]. In this case, the patient is said to have mixed urinary incontinence (MUI). The involuntary leakage of urine that is preceded by urgency is termed urgency urinary incontinence (UUI) and is a distinct problem.

SUI can be demonstrated during routine clinical examination when the patient is asked to cough, or it is demonstrated during pad testing or filling cystometry. Cystometry is performed to exclude the presence of any other functional bladder condition, including detrusor overactivity (DO) that leads to UUI, or bladder outlet obstruction (BOO) that can cause an overflow type of incontinence.

During filling cystometry, the patient's bladder is filled using a pressure transducing catheter and the detrusor pressure can be calculated. When a patient develops incontinence (usually during straining) in the absence of any significant rises in detrusor pressure, the patient is said to have urodynamic stress incontinence (USI). For those who demonstrate DO (non-volitional detrusor contractions) that is associated with urinary leakage, a different treatment algorithm is followed, which aims to attenuate the sensory and motor pathways.

1.1.2 Prevalence

Currently, depending on which method is used to quantify urinary incontinence, the prevalence of the condition can vary widely. At present, there is no consistently used standardised epidemiological definition for SUI. The majority of studies, which assess the epidemiology of SUI use population-based designs and use questionnaires in an attempt to qualify the patient's self-assessment of the condition. Few of these self-report surveys have been designed specifically for this context and therefore poor inter-study reliability exists. More recently, other devices have emerged for this use, including the International Consultation on Incontinence Modular Questionnaire (ICIQ), which includes various patient reported outcome and health related quality of life (HRQoL) domains and has been validated in a variety of study populations [3].

The 5th International Consultation on Incontinence [4] provides the most robust review in this area and demonstrates that 25-45% of women suffer occasional urinary leakage, whereas 10% have incontinence at least weekly. Furthermore, of those with incontinence, SUI accounted for 50% of cases, MUI affected between 7.5-25%, while UUI represented 1-7%. Meanwhile, results of the population survey based Boston Area Community Health (BACH) study, reported the prevalence of SUI in women to be 26.4% [5], while SUI was reported in 44% of women studied in the multinational Epidemiology of Lower Urinary Tract Symptoms Study (EpiLUTS) [6].

1.1.3 Aetiology

There are several factors, which correlate with the propensity to develop SUI in women. However, it is notable that the majority of studies responsible for these data are cross-sectional in design and therefore provide limited evidence of causation. Longitudinal studies would enable temporal associations to be made, however there is a relative paucity of such study designs in the literature.

1.1.3.1 Age

There is a steady increase in the development of urinary incontinence in general with age as demonstrated in figure 1.1.1. A distinct peak exists at the time of the menopause, as demonstrated in the widely reported EPINCONT study (Epidemiology of Urinary Incontinence in Nord-Trøndelag) [7]. As compared with the prevalence of UUI and MUI, which continue to rise with age, SUI tends to decrease beyond the 5th decade. The Nurses' Health Study (NHS) [8] mirrored these findings, demonstrating that SUI decreased over a 2 year observation period, while both MUI and UUI increased. The decrease in prevalence seen for SUI is that UUI is proportionally more common in older age groups and this is more likely to be reported as a bothersome symptom than is SUI [7]. The issues associated with these cross-section studies is that confounding factors, such as childbirth, obesity and age related co-morbidities can also contribute to the propensity to develop SUI.

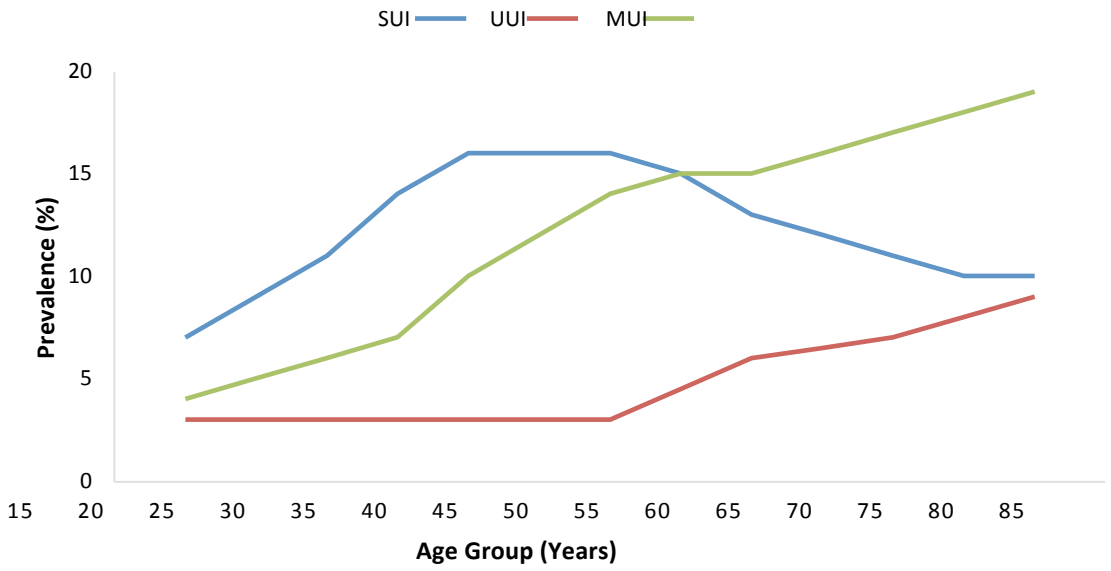


Figure 1.1.1. ***Prevalence of SUI, UUI, and MUI by age***

Value estimates of pooled data from epidemiological studies [9] Figure re-drawn with permission from Reynolds, W.S., R.R. Dmochowski, and D.F. Penson, Epidemiology of stress urinary incontinence in women. Curr Urol Rep, 2011. 12(5): p. 370-6.

1.1.3.2 Parity and vaginal delivery

Stress urinary incontinence is estimated to affect 40-59% of pregnant women; its prevalence and severity increase during the term, with 15-30% of women report SUI during the first post-partum year [4]. The results of the EPICONT study show that the risk of developing SUI is greater for pregnant women aged 20-30 [7]. Caesarean delivery seems to be protective against this problem (OR=0.56) [10]. Following delivery, Thom *et al* [11] found the mean prevalence of SUI during the initial 3 months was 24.6%, while others have reported that the risk of women developing post-partum SUI is greater in women with SUI during pregnancy, even after 3 years has lapsed following delivery [12].

While many early studies suggested that a threshold exists for parity, beyond which no further significant increased risk of developing SUI occurs [13, 14], results of the EPICONT study demonstrates that increasing parity is associated with an increased risk of developing incontinence, particularly in younger age groups (RR=3.3) [15].

1.1.3.3 Obesity

A raised body mass index (BMI) is a significant independent predictor of the propensity to develop incontinence. Systematic reviews of the contemporary literature consistently demonstrate positive correlation between BMI and urinary incontinence [16, 17] and this evidence is based on both longitudinal and cross-sectional data. Data from the EPICONT study [15] also demonstrate that the prevalence of all forms of urinary incontinence rise; MUI showing the greatest increase with a raised BMI (figure 1.1.2). However, it is important to note that age is also associated with a raised BMI with a mean increase in 3 points from age 30 to 60 [16].

Obesity increases the intra-abdominal pressure, which predisposes the patient to develop stress incontinence, highlighted by urodynamic data within the context of the PRIDE trial [18]; the same trial demonstrating improvement or resolution of SUI correlating with the degree of weight loss. This finding is supported by data from case series, including patients undergoing surgical weight loss regimens [19].

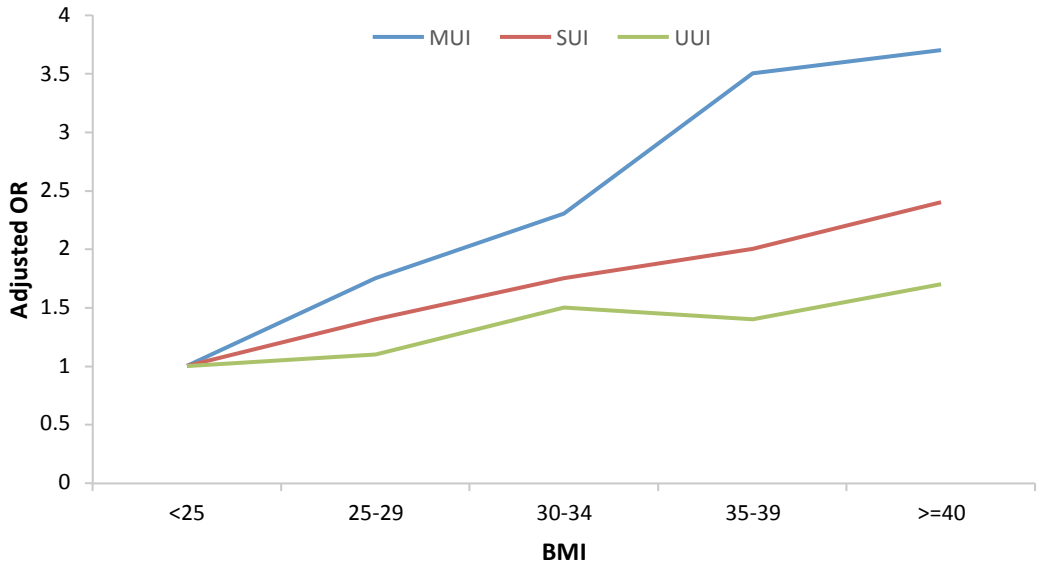


Figure 1.1.2. ***Grouped odds ratios of SUI, UUI, and MUI according to BMI***

Figure re-drawn with permission from Rortveit, G., Y.S. Hannestad, A.K. Daltveit, and S. Hunskaar, Age- and type-dependent effects of parity on urinary incontinence: the Norwegian EPINCONT study. Obstet Gynecol 2001;98:1004-10.

1.1.3.4 Family history

The results of the EPICONT study suggests that genetic factors may be involved in the development of SUI. Daughters of mothers who have SUI had a greater risk of developing SUI, as did siblings of those affected (RR=1.5 and 1.8 respectively) [20]. Although environmental factors inherently are involved in this context, results of twin registry studies demonstrate strong evidence for genetic factors to play a part [21] and several genes, which are associated with connective tissue remodeling have been identified as being associated with the condition [22]

1.1.4 Health Economics

SUI is responsible for a large proportion of health care spending. As a consequence of an ageing population, these costs are likely to rise significantly over the next 20 years [23]. The management costs of SUI was estimated to occupy 0.3% of the entire UK National Health Service (NHS) budget [24], while in the United states, the direct costs of SUI was estimated at $13.1bn in 1995, which is greater than that spent on the investigation and treatment of breast cancer [25], although the actual treatment costs represent less than 10%; the remainder relating to clinic visits and containment products [26].

1.1.5 Quality of Life (QoL)

SUI is a common and debilitating condition. Although it is not a life threatening problem, it can significantly impact upon the patient's QoL. Patients avoid certain physical and social activities in order to minimise leakage. Health questionnaires also consistently demonstrate negative outcomes in the domains of psychological wellbeing and anxiety, such as those included in the EpiLUTS study [27]. Although SUI ranks highly as a frequent cause of depression and anxiety, it is not associated with mental health problems to the same degree as other storage symptoms, such as urinary urgency, nocturia and urgency incontinence [28]. This is almost certainly due to the unpredictable nature of storage symptoms as compared to SUI, which a patient can prevent to some degree by avoiding precipitating situations.

1.2 Pelvic organ prolapse

1.2.1 Definitions

Pelvic organ prolapse (POP) is defined by the International Continence Society (ICS) as "the symptomatic descent of one or more of: the anterior vaginal wall, the posterior vaginal wall, and the apex of the vagina (cervix/uterus) or vault (cuff) after hysterectomy" [2]. Patients with the condition are often asymptomatic, however up to 6% of women over the age of 20 suffer from symptomatic pelvic organ prolapse [29]. Furthermore, data has demonstrated that the relative prevalence of the disorder increases by up to 40% per decade in a cross-sectional study of 1004 US women [30]. Symptoms usually occur when the level of the prolapse extends beyond the hymen and are made worse with gravity. Symptoms can result from the prolapse itself and patients complain of the feeling that 'something is coming down' or experience a dragging sensation. Others describe backache or experience bladder or bowel symptoms and POP frequently co-exists with SUI in up to 30% of cases [34]. Patients describe dyspareunia and may suffer anxiety as a result. Occasionally, patients describe having to manually reduce their prolapse, which is generally encountered in the elderly population.

The location of the prolapse has previously been referred to as involving a particular organ, for example cystocele (bladder), rectocele (rectum), or enterocele (small bowel). As there is no guarantee that these structures are involved in the prolapse, it is more appropriate to refer to the segment of the lower reproductive tract that is associated with weakness. These are described as compartments and three exist:

- Anterior vaginal wall – which commonly involves the bladder and/or urethra.
- Posterior vaginal wall – which commonly involves the rectum or small bowel.
- Apex of the vagina – which involves the cervix or uterus.

  Vault – which is termed following a hysterectomy.

1.2.2 Prolapse classification

The patient should be examined in an appropriate position, which best demonstrates the location and severity of the prolapse. This is best observed with the patient in an appropriate position, which replicates their symptoms. The hymen, a fixed point and acts as a reference point for descriptive purposes. A variety of classification systems have been used to describe POP. In contemporary practice, the pelvic organ prolapse quantification (POP-Q) system is used (figure 1.2.1). The POP-Q score was developed to overcome the problems with validation and objectivity associated with other forms of classification systems [31, 32]. The POP-Q system was initially described by Bump *et al* [33], to describe defects relative to the hymenal remnants and are further staged by the location of the distal most portion of the defeac)t: Stage 0 – No demonstrable prolapse.

b) Stage I – Distal most portion of prolapse is >1cm above the level of the hymen.
c) Stage II – Distal most portion of prolapse is <1cm proximal or distal to the level of the hymen.
d) Stage III – Distal most portion of prolapse is >1cm below the level of the hymen.
e) Stage IV – Demonstrates complete eversion of the total length of the lower genital tract.

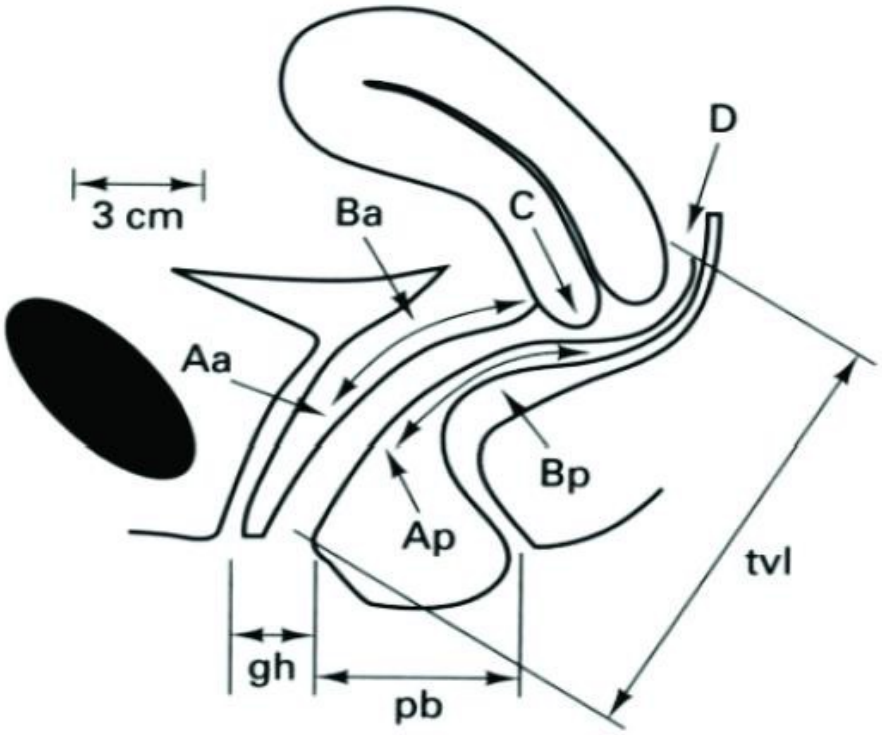


Figure 1.2.1. ***Pelvic Organ Prolapse Quantification System (POP-Q)***

Point Aa: Midline of vaginal wall, 3cm proximal to the external urethral meatus (EUM).

Point Ba: Most distal position of any part of the upper anterior vaginal wall from the vaginal cuff or anterior vaginal fornix to point Aa.

Point C: Distal most edge of cervix or leading edge of the vaginal cuff.

Point D: Posterior fornix in a woman with a cervix.

Point Bp: Distal most position of any part of the upper posterior vaginal wall from cuff/fornix to point Ap.

Point Ap: Point located in midline of posterior vaginal wall 3cm proximal to the hymen.

Genital hiatus (GH): measured from middle of the EUM to the hymen in the posterior midline.

Perineal body (PB): Measured from posterior margin of the genital hiatus to mid-anal opening.

Total vaginal length (TVL): greatest depth of vagina when Point C or D is reduced to its normal anatomical position.

1.2.3 Prevalence

Many cases of prolapse are completely asymptomatic and therefore estimates of the prevalence of the condition vary depending on whether diagnosis is made on symptoms or examination findings. Clearly, age is an important factor and therefore studies that include a wider age range provide a more accurate estimate of prevalence. For studies that use a symptomatic definition of POP, such as a feeling of pressure or bulging demonstrate a prevalence of between 5-10% [34, 35]. Meanwhile, a much higher prevalence (30-50%) is estimated in studies that define POP using a pelvic examination or the POP-Q score [36-38]. The anterior compartment is the most frequently affected by POP, followed by the posterior compartment as described by studies, which use the POP-Q system. The anterior compartment is affected almost twice as often as the posterior compartment [36, 37].

The incidence of POP has been estimated in the context of the WHI Oestrogen Plus Progestin Trial [36], whereby 412 women underwent a standard pelvic examination biannually over an 8 year period. The incidence of anterior, posterior and apical prolapse over the course of the study was 9%, 6% and 2% respectively. The annual rate of prolapse surgery in the UK is estimated at 0.16% in longitudinal studies of patients <40 years of age at baseline [39], while one US study reported an annual incidence of 0.5% for patients aged between 70-79, with an estimated lifetime cumulative risk of surgery for prolapse of 11% [40].

1.2.4 Aetiology

1.2.4.1 Pelvic surgery

Hysterectomy is regarded as increasing the risk for the development of POP, however the lack of longitudinal studies to demonstrate a significant positive temporal association are few. A Swedish cohort study, Altman *et al* reported that 3.2% of women who have undergone a hysterectomy underwent POP surgery, compared to 2% of those who had not undergone a hysterectomy. This would correspond to a relative risk of 1.7 (95% CI, 1.6-1.7). Furthermore, those who underwent a vaginal hysterectomy were at a greater risk for subsequent POP surgery (hazard ratio 3.8; 95% CI, 3.1-4.8) compared with non-hysterectomized controls. Meanwhile, findings

from Mant *et al* [39] in the longitudinal Oxford Family Planning Association Study corroborated these results and demonstrated that the relative risk for the development of POP following a hysterectomy was 5.5 (95% CI, 3.1-9.7) if the surgery was performed for prolapse compared with for other benign conditions. Furthermore, other forms of gynaecological surgery are associated with the subsequent development of POP, including rectopexy for rectal prolapse (odds ratio 3.1; 95% CI, 1.4-6.9) [41], gynaecological surgery in general (odds ratio 3.9; 95% CI, 1.8-8.8) [42], while the Burch colposuspension is associated with a 30% risk of subsequent vault or posterior compartment prolapse [43]. The risk of requiring a further operation in patients having POP surgery following a failed repair has been estimated at 17%, compared with a risk of 12% for those undergoing an initial procedure [44].

1.2.4.2 Parity and vaginal delivery

The Oxford Family Planning Association study [39] demonstrated that childbirth was the single strongest risk factor for the development of POP in women <59 years of age; a risk that increased with each subsequent delivery. Rortveit and co-workers [34] demonstrated that the odds ratio for the development of POP for women with one vaginal delivery was 2.8 (95% CI, 1.1-7.2), two deliveries OR 4.1 (95% CI 1.8-9.5) and three or more deliveries OR 5.3 (95% CI, 2.3-12.3) as compared to nulliparous women. Several studies suggest that caesarean section has a protective effect on the subsequent development of POP. Uma and co-workers demonstrated that caesarean section led to a significant risk reduction in POP surgery compared to vaginal delivery (odds ratio 0.3; 95% CI, 0.05-0.55), findings, which are supported in other case-control studies [45]. Meanwhile, findings from other studies have suggested that in the long-term, there is a negligible effect on the development of POP [46]. In the largest cohort study of women undergoing delivery by caesarean section (n=33,167) and an age matched control group undergoing vaginal delivery (n=63,229) over a 10 year study period, Leijonhufvud found an increased risk of subsequent prolapse surgery for women in the vaginal delivery cohort (hazard ratio 9.2; 95% CI, 7.0-12.1) compared to the caesarean section cohort (figure 1.2.2) [47]. This demonstrates that for women

who delivered vaginally, the incidence for prolapse surgery steadily increased, to reach a peak in excess of 25% towards the third decade, while women who delivered by caesarean section demonstrated only a slight increase in incidence of POP surgery over the same time frame. As compared with SUI, the pregnancy itself does not seem to be a significant risk factor in the development of POP [48].

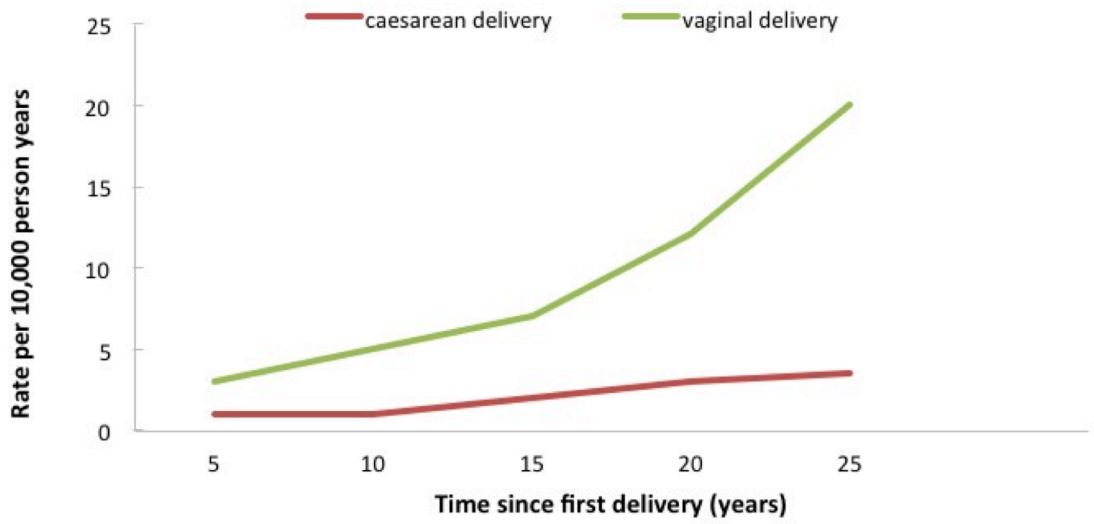


Figure 1.2.2. ***Rate of POP surgery by mode and timing of delivery***

Re-drawn with permission from Leijonhufvud, A., C. Lundholm, S. Cnattingius, F. Granath, E. Andolf, and D. Altman, Risks of stress urinary incontinence and pelvic organ prolapse surgery in relation to mode of childbirth. Am J Obstet Gynecol 2011;204:70 e1-7.

1.2.4.3 Obesity

Despite obesity being strongly associated with the development of SUI (figure 1.1.2), there is a much weaker correlation with POP. Several studies have demonstrated an increase in the symptoms, which obese patients with POP experience, including one study by Washington *et al* [49] in a cross-sectional assessment of obese and non-obese women. Obese women did not demonstrate a significantly increased incidence of stage II or greater POP, but did suffer with more severe symptoms.

1.2.4.4 Connective tissue diseases

Disorders such as Ehlers-Danhlos and Marfans syndrome have been associated with a greater propensity towards the development of POP, with estimates of 75% and 33% for each disorder respectively in a study of 8 patients with Ehlers-Danhlos and 12 with Marfans [50].

1.2.4.5 Family history

The Swedish twin registry provided heritability estimates of 43% for prolapse surgery in the cross-sectional survey based study of 25,364 twins aged 20-46 [21]. Meanwhile, other controlled studies have demonstrated that the unadjusted odds ratio is between 2-3 for first-degree relatives of POP and/or herniae sufferers [45, 51, 52]. McLennan *et al* [51] demonstrated that those with a stage III-IV prolapse have a greater familial component than those with stage I-II POP. This is consistent with findings of other studies, which suggest an earlier onset of POP in familial cases [53]. The authors conclude that this risk is reduced after adjusting for other confounding factors, however it still persists.

1.2.5 Health economics

The annual treatment cost for POP surgery in the US was estimated at $1,012million in 2001; hospitalization was responsible for 71% of this cost, while physician services formed the remainder [54]. Subramanian and colleagues [55] estimated the admissions costs of POP surgery in Germany, France and England. In 2009, the admissions for POP surgery accounted for 10.4%, 16.7% and 16.9% of all female

genital surgery in Germany, France and England respectively. The costs were €144,236,557, €83,067,825 and €81,030,907 for each country respectively, highlighting a significant burden for the disease across the three countries.

1.2.6 Quality of life (QoL)

QoL questionnaire surveys of patients with POP consistently demonstrate that the impact of the disease extends beyond the symptoms resulting from the prolapse itself and can affect psychological, social and sexual function.

1.3 Pelvic floor anatomy

The reasons behind the laxity of pelvic floor tissues that can lead to SUI and POP is still poorly understood. The pelvic floor itself is a supporting structure consisting of muscles, fascia and connective tissue (figure 1.3.1). Appropriate support depends on the complex function of these structures and their respective contributions are discussed below. There are significant inconsistencies in the terminology used to describe the vital pelvic floor components and consensus texts such as the 5th International Consultation on Incontinence (ICI) aim to avoid some of these discrepancies. These suggested terminologies by the ICI are used herein.

1.3.1 Levator ani muscle complex

The levator ani is formed by three separate components. This group of muscles is responsible for the greatest level of pelvic organ support. The action of type I muscle fibres maintains the resting closure of the urogenital hiatus. The three different components are the pubococcygeus, the puborectalis, and the ileococcygeus. A dense fibrous line, the arcus tendineus levator ani (ATLA) runs from the pubic ramus to the ischial spine and provides an attachment from which components of the levator ani arise.

The pubococcygeus is formed at the inner surface of the pubic bones and is sub-divided into three components, which are described according to their respective attachment sites. The pubovaginalis terminates at the lateral vaginal wall, the puboperinealis attaches to the perineal body, while the puboanalis attaches to the anus.

The puborectalis arises from the pubic bone and terminates just above the anal sphincter, passing behind the anorectal junction. The ileococcygeus arises from the ATLA and attaches to the iliococcygeal raphe in the midline, between the anus and the coccyx. It is the narrowest and most posterior of the levator ani muscle components and at its midline attachments is referred to as the levator plate. This area provides support to the rectum, uterus and upper vagina.

1.3.2 The urogenital diaphragm

The urogenital diaphragm (or perineal membrane) lies below the levator ani. It is a dense fibrous sheet, which spans the anterior portion of the pelvic outlet. The dorsal component of the urogenital diaphragm attached to the perineal body and distal third of the vagina, for which it provides support. The ventral part surrounds the compressor urethrae and urethrovaginal sphincter and provides support to the urethra.

1.3.3 The perineal body

The perineal body lies in the midline between the vagina and anus, just deep to the skin. It is a mass of skeletal and smooth muscle components resulting from the converging fibres of the bulbospongiosus, urethrovaginal sphincter muscles, puboperinealis and external anal sphincter. Anteriorally, the perineal body converges with the urogenital diaphragm.

1.3.4 Parietal and endopelvic fascia

The fascial covering of the striated muscle of the pelvic floor is termed the parietal fascia and is responsible for the attachment of these muscles to the pelvis and to provide a point of insertion for the endopelvic fascia.

The parietal fascia has three condensations:

1. The arcus tendineus levator ani (ATLA) arising from the obturator internus muscle.
2. The arcus tendineus fascia pelvis (ATFP) arises from the levator ani from the ischial spine to the superior pubic ramus.
3. The arcus tendineus rectovaginalis (ATRV) commences at the midpoint of the ATFP and attaches to the perineal body.

The endopelvic fascia consists of loose areolar tissue, which contains blood vessels, nerves and lymphatics. The endopelvic fascia gives rise to several condensations/ligaments that provide a supportive role for the pelvic organs.

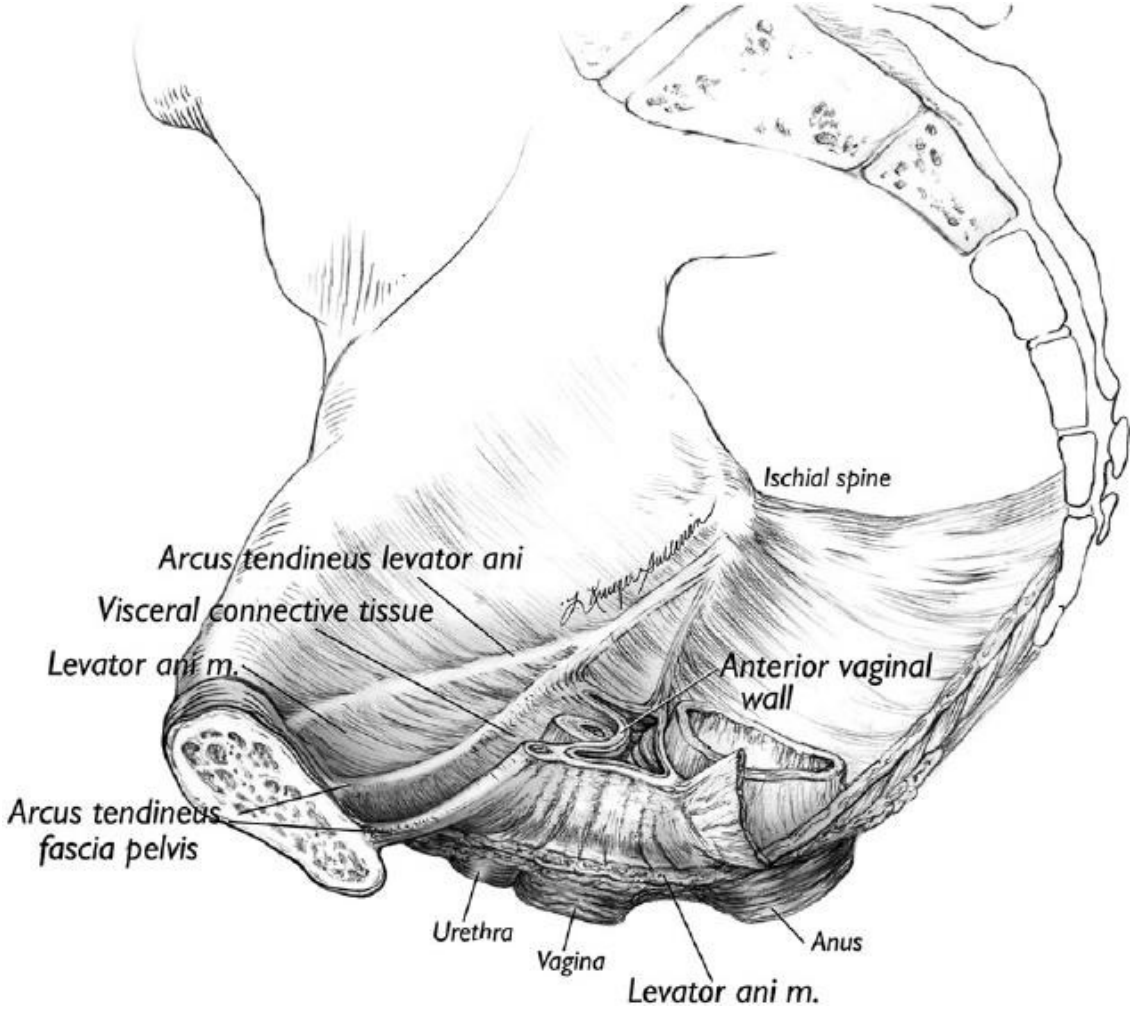


Figure 1.3.1. ***Gross anatomy of the female pelvic floor musculo-ligamentous attachments***
Reproduced with permission from Corton MM. Anatomy of pelvic floor dysfunction. Obstetrics and gynecology clinics of North America. 2009 Sep: 36:401-19.

1.3.5 Ligaments

- **Uterosacral ligaments** – condensations of the endopelvic fascia, which arise from the upper vagina/cervix and attach to the sacrum.
- **Cardinal ligaments** – condensations of the endopelvic fascia, which arise from the upper vagina/cervix and attach to the pelvic sidewall
- **Round ligament** – extends from the uterine cornu to the labia majora, via the inguinal canal. It consists predominantly of smooth muscle and has minimal supportive function, as compared to the uterosacral and cardinal ligaments.

1.3.6 Anterior vaginal wall

A condensation of loose connective tissue connects the anterior vaginal wall to the arcus tendineus fascia pelvis. The anterior vaginal itself wall comprises 3 histological layers: mucosa, muscle and adventitia. During colporrhaphy, it is the muscular and adventitial layers that undergo plication.

1.3.7 Posterior vaginal wall

A condensation of loose connective tissue connects the posterior vaginal wall to the arcus tendineus rectovaginalis. The upper portion of the posterior vaginal wall itself is attached to components of the uterosacral and cardinal ligaments, which provide structural support.

1.3.8 Levels of support

Support for the vagina and uterus are dependent upon the structure and function of the above connective tissues. A system to explain an anatomical basis for pelvic floor disorders based on the dysfunction of these connective tissue structures was proposed by DeLancey [56]. Here, the uterus and cervix attach to the pelvic sidewalls by connective tissue parametrium (uterosacral and cardinal ligaments) and paracolpium respectively. This system comprises 3 levels:

- Level I – The parametrium and paracolpium.
    - Defects usually result in apical prolapse or enterocoele.
- Level II – The attachments of the anterior and posterior vaginal walls to the pelvic sidewall.
    - Defects usually result in anterior or posterior vaginal wall prolapse or SUI.
- Level III – The attachments of the lower vagina, urethra, pubovaginalis, urogenital diaphragm and perineal body.
    - Defects usually result in descent of the perineum, a low rectocele or problems defecating.

1.3.9 Female continence structures

Figure 1.3.2 demonstrates the structures that maintain continence in the female. Continence is a complex process and relies not only upon pelvic floor function, but intact central and autonomic nervous systems. A functioning urethral sphincter mechanism must maintain sufficient closing pressure to resist urinary leakage. The mechanism itself consists of striated, smooth muscle and vascular cushions, each of which contribute equally to the resting pressure of the urethra [57]. The urethra is 'sphincter-active' along its entire length in the female, however it is thickest anteriorally and in the mid-portion. The striated muscle component, the extrinsic portion of the sphincter, is composed of predominantly slow twitch (type 1) fibres, which maintain the resting pressure tone of the urethra. Fast twitch (type 2) fibres assist in maintaining continence during sudden increases in pressure. The striated component comprises three muscles; the sphincter urethrae, the compressor urethrae, and the urethrovaginal sphincter.

The **sphincter urethrae** is the innermost component of the sphincter and surrounds the thickest portions of the urethra.
The **compressor urethrae** attaches to the inferior border of the pubic bone.
The **urethrovaginal sphincter** encircles the urethra and lower vagina. It arches over the urethra on its course inferior to the symphysis pubis.

The sphincter is innervated by the pudendal nerve, which arises from the S_2, S_3 and S_4 nerve roots, while efferents originate from Onuf's nucleus in the sacral cord, which is under the influence of the pontine micturition centre (PMC) in the midbrain.

Deep to the extrinsic sphincter lies the urethral smooth muscle, which is continuous with the detrusor. The sphincteric smooth muscle component is under autonomic control and extends four-fifths of the entire urethral length. It comprises an inner longitudinal and outer circular layer.

Deep to the smooth muscle, lies a plexus of mucosal and submucosal vascular cushions, which assist with coaption of the mucosal surfaces, thereby providing a

water-tight seal.

The function of the lower urinary tract is to convert a constant process of urine production (micturition) into an intermittent process of excretion (voiding) to be performed at an appropriate time and place. The urinary bladder possesses compliance (elasticity), such that the pressure inside the bladder does not rise significantly as the volume of urine contained within it increases. In health, the bladder capacity is roughly 500-600 mls and an initial desire to void is usually reached at 200-300mls. Urine storage and voiding are processes controlled by reflex centres in the spinal cord and the PMC, involving both the autonomic and somatic components of the nervous system. The parasympathetic pathway results in bladder (detrusor muscle) contraction and involves nerve roots S2-S4. During normal bladder filling, there are no stimulatory impulses sent to the sacral micturition centre from the midbrain. The sympathetic nervous system inhibits detrusor contraction and is responsible for contraction of the internal sphincter. Therefore, voiding requires the coordinated detrusor contraction and smooth muscle sphincteric relaxation, in addition to reduced resistance at the level of the striated distal (voluntary) sphincter mechanism. Any abnormalities of this process would lead to problems during the storage (filling) or the voiding phase.

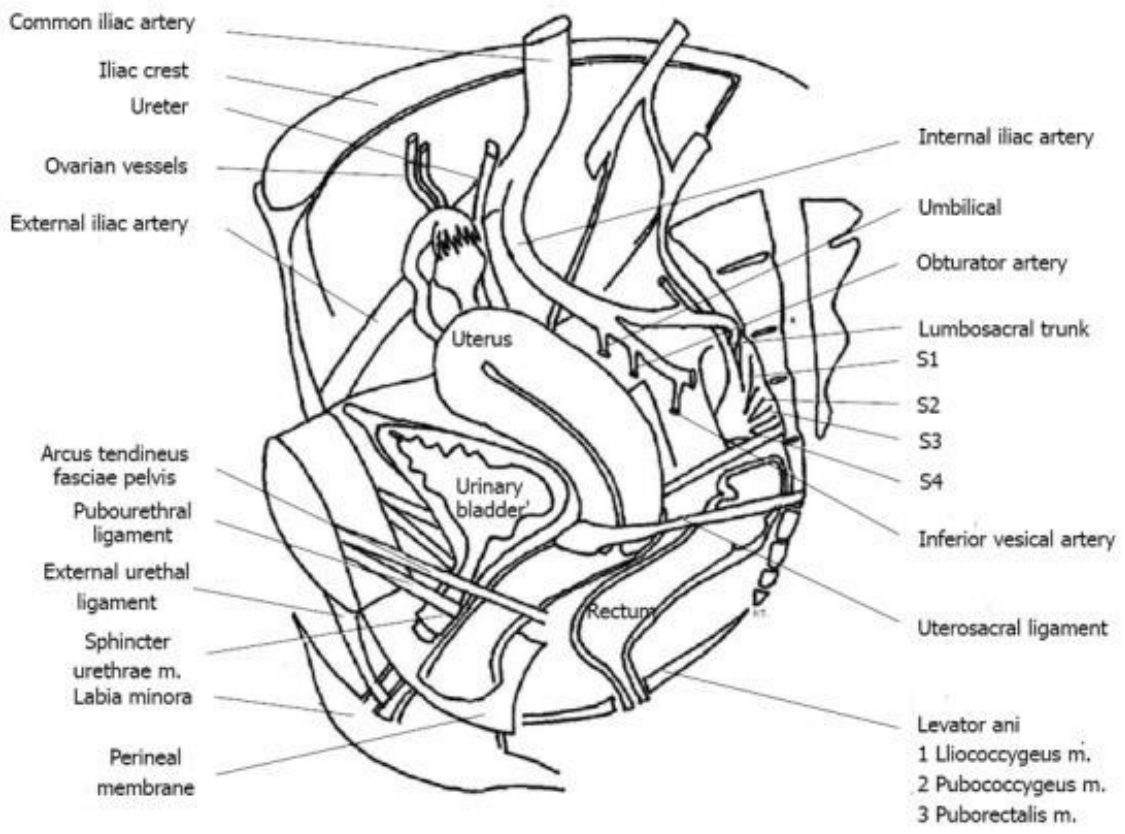


Figure 1.3.2. ***Continence structures of the female pelvic floor***

Reproduced with permission from Thaker H, Sharma AK. Regenerative medicine based applications to combat stress urinary incontinence. World Journal of Stem Cells. 2013;5(4):112-123.

1.4 Pelvic floor dysfunction

1.4.1 Mechanism for the development of SUI

Several factors are involved in continence. The exact mechanism behind this is related to the connective tissue of the pelvic floor, urethral sphincter and the central and peripheral nervous system. The combined action of these mechanisms is responsible for the prevention of urinary leakage during effort or exertion. Therefore, continence relies upon the following factors:

- Striated sphincter under pudendal nerve innervation
- Healthy urethral mucosa and sub-mucosa
- Intact and functioning urethral smooth muscle
- Intact vaginal wall support

At present, there are two prevailing theories, which attempt to explain the development of SUI; urethral hypermobility, and intrinsic sphincter deficiency. These two pathophysiological mechanisms are not completely dichotomous, rather they represent a spectrum on which patients may have features of both [58].

1.4.1.1 Urethral hypermobility

Enhorning [59], was one of the first to suggest that SUI develops due to the urethra descending relative to the pelvic outlet, resulting in abnormal transmission of the raised intra-abdominal pressure during exertion (figure 1.4.1). Elevation of the proximal urethra to a retropubic position as in a Burch colposuspension or pubovaginal sling utilizes this principle in the treatment of SUI. The institution of this early theory in the 1960's has several criticisms; several studies have demonstrated that in fact the pressures in the distal urethra are greater than those in the proximal urethra [60] and that patients with distally located proximal urethras can remain continent [61]. This suggests that continence in this context may be related to a more complex mechanism.

Petros and Ulmsten first proposed potential mechanisms for the development of SUI in the early 1990's. Their 'integral theory' [62] involves laxity of the tissues of the pelvic floor that are involved in urethral 'kinking' and compression in order to

maintain continence. The muscles involved in this process are those that surround the urethra relative to the vagina; the pubococcygeus anteriorally, the levator plate posteriorally and the longitudinal muscle of the anus inferiorly. When this mechanism fails, due to laxity of the anterior vaginal wall and the ligaments surrounding the urethra, for which the pubococcygeus muscle is unable to compensate, the urethra is unable to close sufficiently.

DeLancey [56] described the 'hammock theory', hypothesizing that an increase in the intra-abdominal pressure becomes translated to the urethra, which in health, becomes compressed against the endopelvic and pubocervical fascia (figure 1.4.2). Therefore, it is this compression that leads to continence, rather than effective urethral kinking.

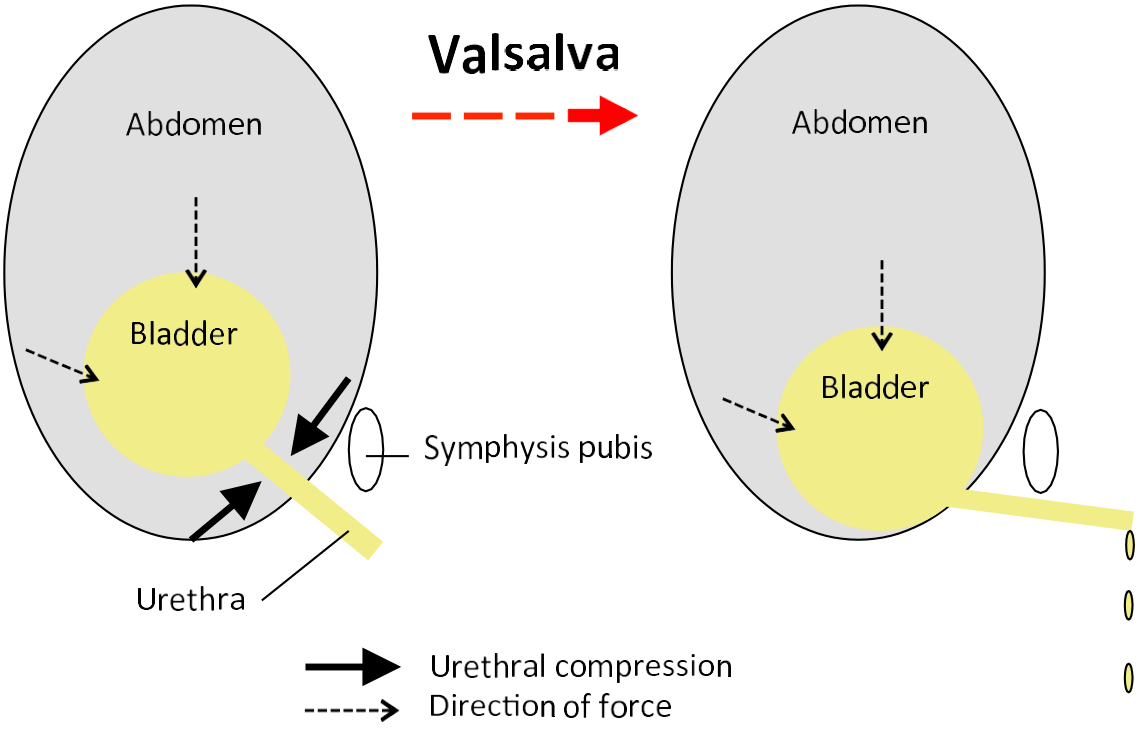


Figure 1.4.1. ***Pressure transmission theory of stress urinary incontinence***

In health, the intra-abdominal portion of the urethra is compressed through effective pressure transmission, while the right image demonstrates loss of this pressure transmission, leading to urinary leakage.

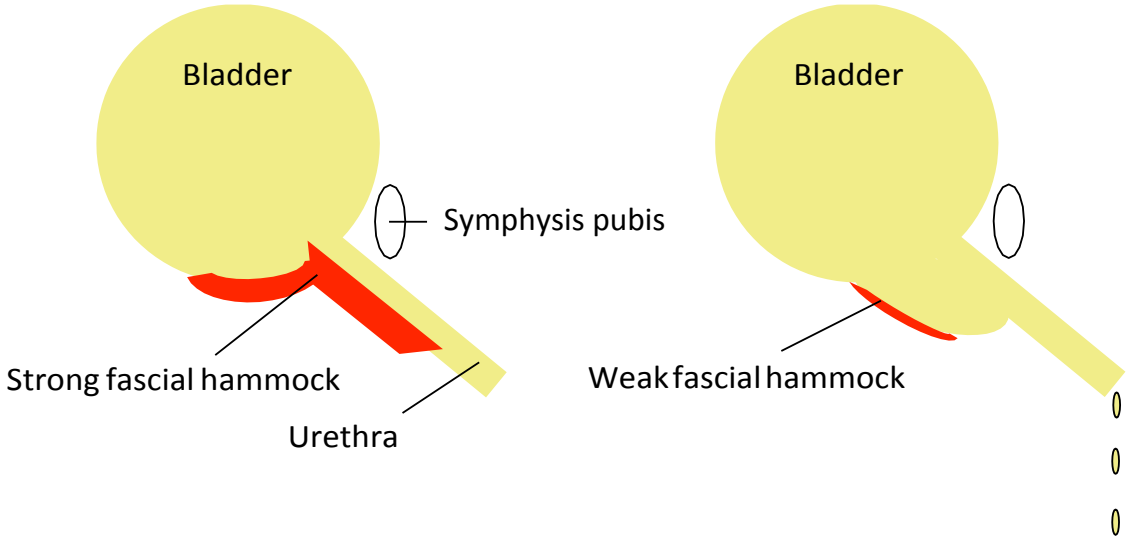


Figure 1.4.2. ***Hammock theory of stress urinary incontinence***

Left image demonstrates strong fascial support that allows compression of the urethra. Right image demonstrates poor support, which does not facilitate urethral compression against the ATFP.

1.4.1.2 Intrinsic sphincter deficiency

The striated muscle comprised of fibres of the compressor urethrae and urethrovaginal sphincter provides a degree of voluntary control, whereas the inner smooth muscle layer occupies the two-thirds of the proximal urethra. Intrinsic sphincter deficiency (ISD) is a mechanism for the development of urinary incontinence that was coined from the observations of voiding video-cystometry findings in patients who have previously undergone procedures aimed at the treatment of urethral hypermobility [63].

The concept ISD was originally described by Edward McGuire in the 1970's [64, 65]. At the time, Enhorning's theory for hypermobility was the prevailing theory for the development of SUI. Using video-urodynamics, McGuire identified a group of patients who had stress leakage despite having a non-mobile bladder neck. This type of incontinence was classified as Type III in his classification system which was later modified by Blaivas and Olsson [66]. Causes of ISD include childbirth related injuries, including ischaemia, neural injury, or other iatrogenic injury. ISD is commonly associated with previous surgery for stress incontinence, which has resulted in significant peri-urethral fibrosis with an eroded urethral tape. Damage to the

sphincter through other forms of trauma including radiotherapy is an important cause. Causes of neurogenic related ISD include conus injuries of the spinal cord and/or the cauda equina, or less frequently the pelvic nerves.

ISD is associated with more pronounced symptoms and is considered more challenging to treat than urethral hypermobility. Operations that were designed to correct urethral hypermobility (e.g. Burch Colposuspension, Marshall-Marchetti-Krantz (MMK) procedure) are known to have a poor outcome in patients with low maximal urethral closure pressure (MUCP) (<20cm H_2O) [63, 67]. The MUCP is produced by the high-pressure zone of the mid-urethra. If this area is deficient (and the pressure is below 20cm H_20), ISD is said to exist [67]. The valsalva leak point pressure (VLPP) is commonly used as an indicator of ISD instead of an MUCP [68]. VLPP is the abdominal pressure at which the patient leaks urine whilst performing a Valsalva maneuver. Despite evidence to suggest that MUCP and VLPP do not correlate, a MUCP of <20cm H_20 has been equated with a VLPP of <60cm H_2O [69].

Various clinical tests are described to enable a diagnosis of urethral hypermobility, including the Q-tip test, the Marshall-Bonney or Ulmsten tests. More recently, a further clinical test has been proposed to identify ISD in women without the need for a urodynamic study. The posterior vaginal wall pull down manoeuvre [70] described by Thubert *et al* is a simple clinical test that involves gentle downward traction of the posterior vaginal wall provided by a split speculum performed with the bladder filled with 400mls of saline in a supine position. A positive test (leakage demonstrated during the procedure) was shown to correlate with ISD (MUCP <20cm H_2O) with a positive predictive value of 94.67%.

1.4.2 Mechanism for the development of POP

While tail movement is the main function of the levators in animals, in humans, the pelvic floor connective tissue provides support to the pelvic organs. The relative contribution of each individual component is a matter of some debate, however at rest, it is the action of tonic contraction, which occurs to effectively allow the urogenital hiatus to close.

POP can develop as a result of the loss of levator tone, which manifests itself particularly during a Valsalva manoeuvre. Loss of levator tone can result either as a consequence of direct injury to the muscles or ligaments, or indirectly following denervation of the nerve supply to the pelvic floor. The latter can occur during prolonged labour, particularly during the second stage, whereby stretch compression of the sacral nerve roots can result in transient or permanent neurological impairment. Levator ani muscle biopsies of patients with POP do demonstrate denervation during histological examination [71].

The mechanism of birth injury has been demonstrated through magnetic resonance (MR) and more recently by 3-D ultrasound imaging. Women with levator avulsion defects as shown during ultrasound investigation were twice as likely to demonstrate POP than those who were not shown to possess levator defects (RR 1.9; 95% CI, 1.7-2.1) [72]. Van Delft demonstrated that following the first vaginal delivery, 21% of women demonstrated a levator avulsion, and this was associated with significantly worse outcomes for pelvic floor muscle strength than women without avulsion defects [73]; findings, which were supported by evidence from DeLancey *et al* [74].

Atrophy of skeletal muscle can also occur with advancing age, and although higher POP stages are often seen in older patients, the evidence to support that this occurs as a direct result of atrophy of the levator ani is lacking in the literature [75].

Whatever the aetiology, it is clear that loss of the levator tone is associated with a greater size of the levator hiatus and this correlates with POP [76]. The result of this is that the vaginal axis is lost and the levator support is inadequate to resist the downward pressure on the pelvic organs that occurs during abdominal straining. Subsequently, a greater stress is placed on the connective tissue components of the levator shelf and if this excessive strain is maintained over a long period of time, clinical features of POP may become obvious (figure 1.4.3) [77]. Whether or not the connective tissues become excessively stretched or indeed tear, is a matter of some debate and it is likely that a combination of the two occurs (figure 1.4.4) [78]; findings,

which have led surgeons to re-approximate the fibromusclar wall of the vagina to the surrounding supportive structures [79].

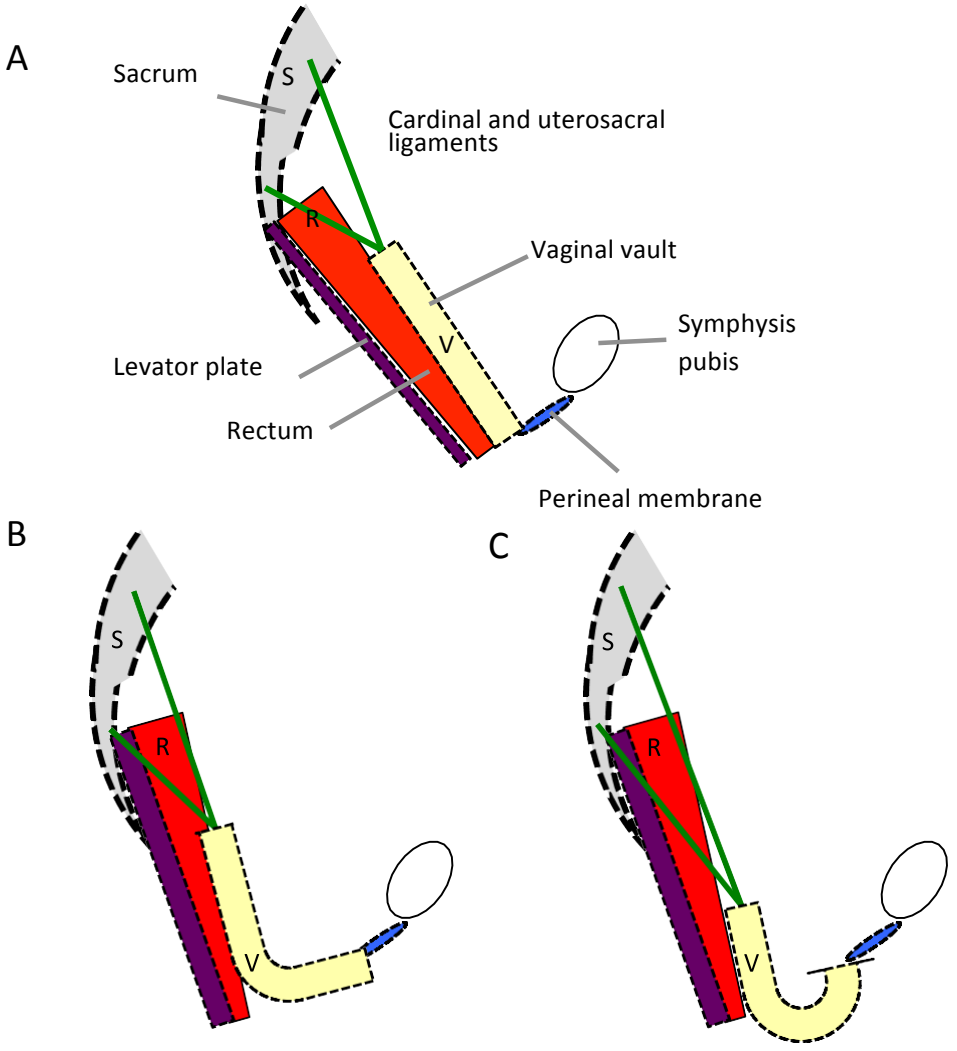


Figure 1.4.3. ***Mechanisms for the development of pelvic floor dysfunction***

A) demonstrates normal pelvic floor structures. Pelvic organs are supported by the levator plate and supportive ligaments. B) demonstrates loss of the levator axis, which leads to the downward displacement of pelvic organs. C) Further downward displacement and prolapse of the vagina due to weakening of supportive ligaments. S = Sacrum, R = Rectum, V = Vagina.

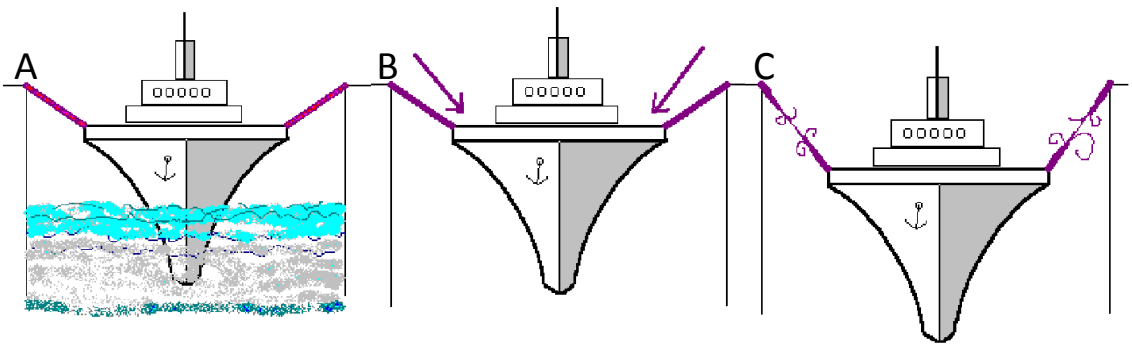


Figure 1.4.4. ***Paramore's theory of pelvic floor dysfunction***

A) demonstrates the pelvic organ structures (ship) supported by the levator group (water) and the suspensory ligaments (ropes). B) demonstrates loss of the levator component, which leads to the downward displacement of pelvic organ structures and stress placed upon the cardinal and uterosacral ligaments. C) demonstrates further weakening and descent of the pelvic organs. Reproduced with permission from Wein et al. Campbell-Walsh urology 10th edition, Elsevier.

1.5 Pathogenesis of pelvic floor dysfunction

Weakness of the pelvic floor connective tissues is a major contributory factor in the development of SUI and POP. While there are patients in whom SUI exists in the absence of pelvic floor weakness (as occurs with ISD), the majority of cases of SUI are associated with connective tissue dysfunction. Connective tissue weakness can occur as a result of changes in the histologic composition of the vaginal wall itself, musculo-ligamentous structural changes or alterations in the composition of extracellular matrix proteins and the associated biochemical components.

1.5.1 The vaginal wall

From inside to out, the vaginal wall itself is composed of 3 distinct layers (Figure 1.5.1):

1. **Mucosa** – composed of the non-keratinized stratified squamous epithelium and the underlying lamina propria.
2. **Muscularis mucosa** – composed of smooth muscle.
3. **Adventitia** – comprising of loose connective tissue.

The outer adventitial layer is thinnest and therefore most of the strength of the vaginal wall is contributed by the inner two layers: the lamina propria and the muscularis. The blood supply to the vagina is provided by the vaginal and uterine arteries, both of which arise from the internal iliac arteries. Venous drainage is achieved from the rich vaginal plexus that lies within the lamina propria and drains into the internal iliac veins.

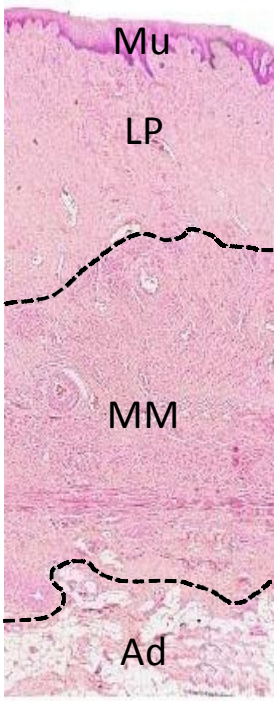


Figure 1.5.1. ***Histology of the vaginal wall***

Mu) Mucosa. LP) Lamina propria. MM) Muscularis mucosa. Ad) Adventitia.

1.5.2 Musculo-ligamentous structural changes

The vaginal wall and supporting structural components can be affected by atrophic changes, which can occur as consequence of the relative lack of oestradiol following the menopause [80]. Studies have demonstrated a deficiency of non-vascular smooth muscle in samples of vaginal tissue taken from patients with POP as compared to patients without POP [81-83], which authors proposed is due to a deficiency of the particular smooth muscle phenotype in these patients, while Takacs *et al* [84] suggest that it is increased vaginal smooth muscle apoptosis that leads to the muscular deficiency. Meanwhile, other authors have concluded that blinded histological analysis of the vaginal wall in patients with and without prolapse does not completely explain these findings [85].

1.5.3 Extracellular matrix proteins

The connective tissues of the pelvic floor are composed of smooth muscle and extracellular matrix (ECM) components, including a ground substance and fibrillar proteins, collagen and elastin. Collagen and elastin contribute most to the tensile properties of the pelvic floor connective tissues and are the most abundant of the ECM components (84% and 13% respectively [86]). Fibroblasts produce the majority of ECM components and can be recognized morphologically by their branched structure and elliptical nucleus. Fibroblasts are involved in the remodeling of the pelvic floor connective tissue through a balance between ECM synthesis and degradation.

Proteoglycans and glycosaminoglycans (GAGs) comprise the ground substance of the ECM and are involved in the remodeling of the matrix components. The remodeling process involves cell migration, proliferation, cell adhesion and fibril organization. GAGs are long unbranched polysaccharide units and synthesized by the Golgi apparatus (except hyaluronan) of cells, which are present in high number in connective tissues, particularly fibroblasts. The GAG, hyaluronan (hyaluronic acid), is perhaps the most important in this context, and is responsible for cell transport and the inflammatory response of the ECM. Proteoglycans consist of GAGs, attached to a protein structure through a tetrasaccharide bridge at the serine residue. These molecules are strongly hydrophilic and can trap water in addition to lubricating collagen fibres, which permits them to glide over one another.

3 distinct groups of proteoglycan exist in the body:

- Hyalectans (lecticans) – larger proteoglycans.
- Small leucine-rich repeat proteoglycans (SLRPs).
- Heparan sulphate proteoglycans – mainly involved in proliferation.

The degradation of ECM components occurs through the group of matrix metalloproteinase (MMP) enzymes, whose homeostasis is dependent upon signals from cytokines and growth factors. Therefore, collagen and elastin expression are tightly controlled in these tissues.

Collagen is responsible for much of the tensile properties of tissues. Collagen is a triple helical protein, which is composed of two identical α-1 chains and a unique α-2 chain;

the amino acids glycine and proline are most prevalent [87] . At present, 28 types of collagen have been described [88].

In the pelvic floor, fibrillar collagens are abundant, with type I and III collagen being the most common [89]. Type I collagen is present in scar tissue and is found in tendons, skin and muscle fibres, while type III collagen is synthesized by immature fibroblasts and is weaker than the type I collagen that is produced by mature fibroblast cells. Collagen I forms thicker fibrils and is therefore present in a greater proportion in those tissues that require a greater tensile strength [90], while a greater proportion of type III collagen is observed in tissues requiring flexibility. Type V collagen is formed by the co-polymerisation of both type I and III collagen to form hybrid fibrils; the properties of which depend upon the ratio of type I:type III collagen. Fibrillar collagen is synthesized through nuclear transcription, followed by translation in the ribosomal subunits and finally modification and fibril formation [91].

Figure 1.5.2 represents the steps in the formation of a collagen fibril. Collagen synthesis begins with turning on the genes, which are associated with the formation of a particular α subtype. Each codes for a specific mRNA sequence, typically with the 'COL' prefix. The mRNA enters the cytoplasm, where it links with ribosomal subunits to begin the process of translation. The signal sequence of the new peptide is recognized by a signal recognition particle on the endoplasmic reticulum (ER), which directs the pre-pro-peptide into the ER itself for post-translational processing. Inside the ER, the signal sequence on the N-terminal is removed (pro-peptide) and hydroxylation of selected proline and lysine residues occurs by the enzymes prolyl hydroxylase and lysyl hydroxylase to aid cross-linking. This step requires vitamin C as a co-factor and is followed by the glycosylation of hydroxyl groups that were placed on lysine residues with either glucose or galactose monomers.

Three of the hydroxylated and glycosylated pro-peptides twist together beginning at the C terminus and di-sulphide bonds form to make the triple helical structure, now termed pro-collagen. This is packaged into a transfer vesicle and transported to the golgi apparatus for one final post-translational modification, whereby oligosaccharides are added and the pro-collagen is destined for the extracellular space. Here, collagen

peptidases cleave the N and C terminal peptides to form tropocollagen, which is assembled into fibrils, using lysyl oxidase, an enzyme that forms aldehyde groups on lysine and hydroxylysine domains. These subsequently become covalently linked between tropocollagen molecules to form the mature fibres that are finally organized into bundles.

SLRPs regulate collagen fibrillogenesis and Decorin deficient mice demonstrate significantly impaired fibrillogenesis with subsequently attenuated tissue tensile strength [92], which is purported to occur due to the impaired ability of crosslinking to occur at the lysyl residues in decorin deficiency. Meanwhile, deficiencies in other SLRPs, such as fibromodulin and lumican result in defective collagen fibrils [93] and gene knock-out mice with fragile skin [94] respectively.

The degradation of collagen occurs mainly through the action of MMPs, which are produced intra-cellularly and released as pro-peptides. Over 20 MMPs are described and the interstitial collagenases (MMP1, MMP8, MMP13, MMP18 and to a lesser extent MMP2 and MMP14) cleave fibrillar collagen, while the gelatinases (MMP2 and MMP9) breakdown the resultant denatured peptides along with type IV collagen and gelatin [95]. The stromelysins cleave ECM proteins, however are unable to cleave fibrillar collagens.

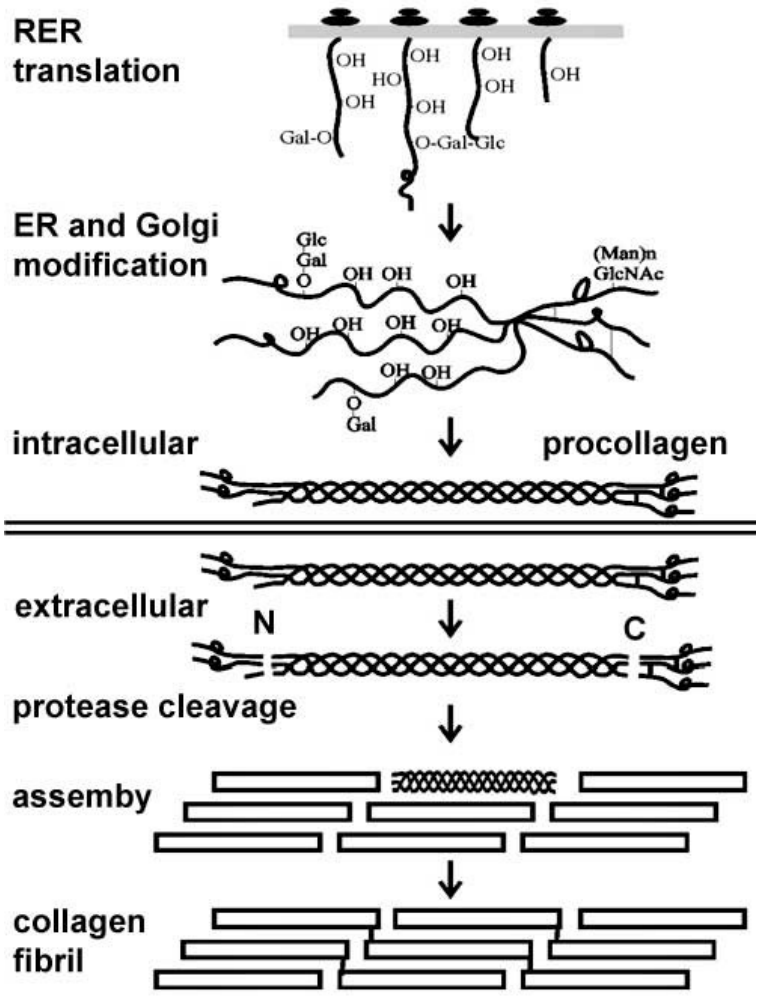


Figure 1.5.2. ***Diagrammatic representation of collagen synthesis***

RER. Rough endoplasmic reticulum. ER. Endoplasmic reticulum. Reproduced with permission from Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell, 4th Ed. 2002. Garland Science. Chapter 19. Cell Junctions, Cell Adhesion, and the Extracellular Matrix.

MMPs are synthesized as pro-peptides and are secreted as pro-enzymes, which require extracellular activation. MMPs are inhibited by endogenous tissue-derived inhibitors of MMPs (TIMPS), of which there exist 4 subtypes. TIMPs are responsive to a variety of cytokine and hormonal influences and can also have an effect on apoptosis, platelet aggregation, cell proliferation and hormone regulation. Certain TIMPs demonstrate different effects on different MMPs.

Elastin is the second peptide responsible for the strength and elasticity of the pelvic floor. Connective tissues that express a greater proportion of elastin demonstrate the ability to stretch and recoil under tensile loading; the tissue returning to a near normal configuration after distension has occurred [96]. Elastin fibres are therefore found in

abundance in the ECM of tissues that are subject to long-standing repetitive strain, such as the pelvic floor, arterial walls, the lungs and skin. The elasticity of this protein is due to the arrangement of alternating hydrophobic and hydrophilic elements, which form β-spirals that can stretch [97].

Elastin fibres are synthesized during the gestational period, terminating shortly thereafter, whereby no further elastin is made except during the pelvic floor remodeling processes that occur following pregnancy [98].

The ELN gene encodes proteins that are rich in hydrophobic amino acids, such as glycine and proline, bounded by cross-linked lysine residues to form the precursor of elastin, tropoelastin. Elastin is made through the linkage of multiple tropoelastin molecules, in a reaction catalyzed by lysyl oxidase. These combine with fibrillin, a glycoprotein that is secreted into the ECM by fibroblasts and provides a scaffold for the deposition of tropoelastin. Elastic fibres are created from the extensively cross- linked elastin and fibillar components formed by the crosslinking of lysine residues.

Owing to the extensive cross-linking that occurs between the lysine residues, degradation of elastic fibres is a slow process in health. Serine proteases, such as neutrophil elastase, cysteine proteases, such as cathepsins L, S and K and MMP2, MMP9, MMP12 can degrade elastin.

1.5.4 Clinical significance

The findings of studies that assess collagen and elastin content in the diseased state as compared to pelvic floor tissues are discussed herewith. The content of these ECM proteins are generally assessed during remedial surgery for POP and SUI. While deficiencies in total collagen or elastin content can be demonstrable, it is difficult to attribute these biochemical changes to deficiencies in either their synthesis or degradation. Therefore, other markers can be used.

1.5.4.1 Collagen content in the diseased state

The total collagen content of certain pelvic floor tissues in patients with SUI appears to be consistently reduced in the vaginal wall [99, 100], periurethral and round ligaments [101, 102]. These findings are consistent in those with POP, where total collagen content is reduced in the parametrium and vaginal apex [103] and uterosacral ligaments [104]. Meanwhile, other studies have demonstrated an increase in total collagen content of the cardinal and uterosacral ligaments [105] in those with POP and/or SUI. These findings however, may reflect a change in the ratio of collagen I to collagen III, with a much greater proportion of collagen III deposited following the tissue remodeling processes that occur in disease [106] and signifies reparative processes in response to overstretching. Many studies do demonstrate a significant increase in the proportion of collagen III [100, 105, 107, 108] and a greater proportion of collagen III has been associated with weaker tissues[109].

However, the difficulty in differentiating between altered collagen synthesis *versus* increased degradation remains. Makinen *et al* [110] overcame this problem, by isolating and culturing the fibroblasts taken from tissue biopsies of patients with POP as compared to those without POP and measuring the collagen production *in vitro*. No significant difference was demonstrated between those with POP and controls; findings which are supported by Chen *et al* [99], who compared those with and without SUI. The use of novel biomarkers to measure collagen metabolism, such as carboxy-terminal telopeptide of type I collagen (ICTP) and carboxy-terminal propeptide of type I procollagen (PICP), which are markers of collagen I breakdown and synthesis respectively have demonstrated increased levels of both in those with SUI, suggesting greater synthesis, following collagen degradation [111]. Other investigators however have failed to replicate these findings in patients with POP and SUI [112].

MMP or pro-MMP levels can be measured to assess the degree of collagen degradation. Jackson *et al* [113] demonstrated a significant reduction in total collagen content and up to four-times higher MMP-1 activity in vaginal epithelia specimens of patients with prolapse versus age matched controls. Other studies have demonstrated increased MMP9 levels in those with POP [100], while Chen *et al* [99] found no significant difference in MMP2 or MMP9 level in patients with POP and SUI, however

reduced TIMP1 levels were present.

The altered collagen metabolism that is seen in these patients could also result form a global collagen dysfunction. Those with Ehlers-Danlos, a genetic condition, which affects the synthesis of collagen have a higher incidence of SUI and POP than those without this collagenopathy [50, 114]. Similarly, women with POP are at a greater risk of requiring hernia surgery [115] and also chronic obstructive pulmonary disease (COPD) [116]. Ulmsten *et al* demonstrated that the skin of women with SUI contained 40% less collagen than those without SUI [102], while Soderberg showed a 30% reduction in the ability of skin fibroblasts to produce collagen in those with SUI as compared with healthy controls [117].

1.5.4.2 Elastin content in the diseased state

Disorders of elastin metabolism are associated with an increased risk of the development of both POP and SUI. Marfan's syndrome is a genetic condition, caused by a mutation on chromosome 15, which encodes the fibrillin; the glycoprotein required for the synthesis of elastic fibres. A similar genetic condition called cutis laxa is associated with mutations in the genes that encode elastin translation. The result of these conditions is that the ECM component of tissues is poorly elastic. This is most obvious in the skin, where once stretched inadequately recoils. These disorders are associated with other systemic conditions that result as a consequence of defective elastic fibre metabolism, such as aneurysmal change, cardio-pulmonary disorders and herniae. Patients with either condition have a high incidence of POP and/or SUI [50]. Other studies have demonstrated a clear reduction in elastin content of pelvic floor tissues for those with POP and/or SUI (Figure 1.5.3) [104, 107, 118]. While the elastin content has been quantified using polymerase chain reaction [119]. Although, the direct measurement of mature elastic fibres is difficult; many studies measure elastin at the mRNA level or quantify tropoelastin, which may not accurately reflect the complex synthesis/degradation of these ECM proteins in such patients and therefore, immunohistochemistry is considered the most accurate measurement [120]. Data from other studies suggest that elastin degradation is more important than elastin synthesis. Systemic elastase activity in patients with SUI has been shown to be over three times greater [121], while alpha-1-antitrypsin, a protease inhibitor, has been

shown to be deficient in peri-urethral tissue specimens of patients with SUI [119]. At low levels, there is reduced inhibition of neutrophil elastase, which is free to break down elastin and is also associated with pulmonary vascular disease and cirrhosis.

In conclusion, the available evidence would suggest that the total collagen content of pelvic floor tissues in patients with SUI/POP is generally low/normal as compared to age matched patients. Perhaps more important than the total collagen content is the collagen I to collagen III ratio – a greater proportion of collagen III is associated with tissues that are remodeling. In this context, it is more likely that increased collagen degradation is responsible rather than defective synthesis. Elastin content of disease pelvic floor tissues is reduced, however, whether this is due to defects along the complex synthetic pathway or due to an increase in the degradation of elastin is not completely clear and it is likely that both contribute to this process.

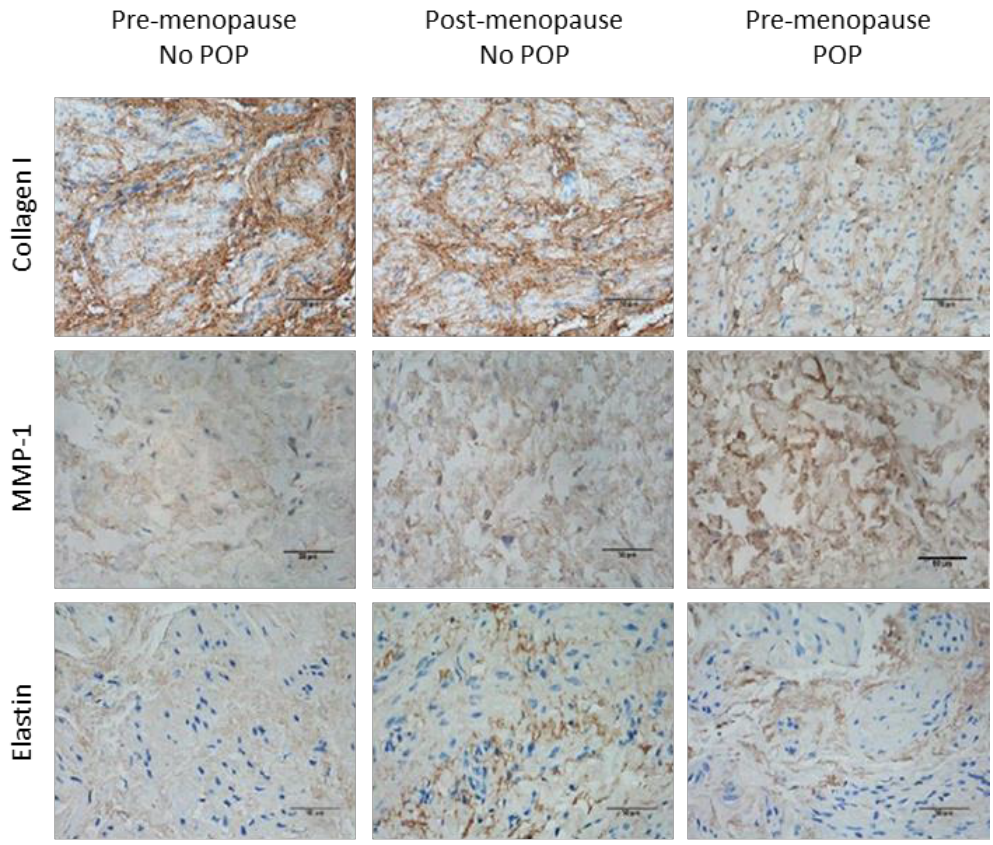


Figure 1.5.3. ***Representative immunohistochemistry images of uterosacral ligament specimens of pre and post-menopausal women with and without POP***

Cells stained blue, ECM proteins stained brown. Collagen I expression is greater in pre-menopausal women than in post-menopausal women without POP, which in turn is greater than those with POP. MMP-1 expression is greater in post-menopausal women with POP, while elastin is expressed at a greater quantity in pre-menopausal women. Reproduced with permission from Aznal, S.S., Meng, F.G., Nalliah, S, Tay, A., Chinniah, K., Jamil, M.F. , Biochemical evaluation of the supporting structure of the pelvic organs in selected numbers of premenopausal and postmenopausal Malaysian women. Indian Journal of Pathology and Microbiology 2012;55:450-455.

1.5.5 Function of fibroblasts in the ECM

Fibroblasts are an important component in the production of extracellular matrix component production in the pelvic floor. These cells are also involved in a variety of other processes in this context. Fibroblasts express endothelin-1, which has a role in the contractility of myofibroblasts. Women with POP demonstrate a reduction in the contractility of vaginal myofibroblasts and lower levels of endothelin-1 expression as compared to those without POP [122]. Furthermore, the vaginal myofibroblasts of primaparous pre-menopausal women appear to demonstrate greater contractility as compared to multi-parous women with POP, as measured using a collagen gel contraction assay [123]. Interestingly, in this study pre-menopausal, multiparous women demonstrate similar values for cell contraction as post- menopausal women with severe POP.

The fibroblasts that are found in patients with POP demonstrate increased proliferative rates and an altered cytoskeleton architecture in response to stretch, which disrupts the ligamentous integrity [124]. Women with POP also demonstrate reduced expression of the mRNA for p53 in the cardinal ligaments [118]. Cells are therefore unable to enter the quiescence phase (Go phase), and therefore cells continue to divide, reducing the production of ECM components. Fibroblasts produce transforming growth factor β1 (TGF- β1), a polypeptide with important roles in wound healing, the immune system in apoptosis. Women with SUI demonstrate a reduction in the level of TGF- β1 in fibroblasts taken from the vaginal wall and this is purported to affect the stimulation of collagen and elastin synthesis [125].

1.5.6 Effect of oestradiol on ECM component production

The sex steroid oestradiol has long been used for the treatment of post- menopausal women with SUI and POP. Oestradiol replacement in women has been demonstrated to increase the local production of both collagen I and collagen III mRNA [126] and also increase the collagen I/collagen III ratio in oophrectomized rats [127]. Oestradiol inhibits MMPs and studies have demonstrated reduction in the levels of MMP and an increase in TIMPs in the para-urethral tissues of women with POP [128]. However, other studies have demonstrated that oestradiol may stimulate collagen synthesis with relatively immature cross-linkages, which can be easily degraded by MMPs. This

leads instead to a total reduction in functional collagen fibres in the ECM [129].

Zong *et al* [130] demonstrated that effective inhibition of MMPs is achieved with oestradiol and progesterone in combination. The co-administration of progesterone and oestradiol in the media of cultured fibroblasts isolated from the arcus tendineus was shown to reduce MMP13 more effectively than either alone [131]. Furthermore, Moalli [132] demonstrated a reduction in the collagen I to collagen III ratio in post-menopausal women, as compared to younger patients. Following treatment with both oestradiol and progesterone, the total collagen I and therefore the collagen I to collagen III ratio was increased. Low serum oestradiol levels are associated with urethral hypermobility in post-menopausal patients [133]

1.6 Biomechanics of the pelvic floor connective tissue

The main function of the pelvic floor connective tissue is to resist increases in intra-abdominal pressure and to effectively recoil following distension. Pressure transmission to the pelvic floor is affected by gravity from the abdominal viscera. Therefore, a patient's posture, habitus and the presence of additional intra-abdominal mass can influence the forces that are transmitted to the pelvic floor. Intra- abdominal pressure can also increase suddenly during coughing, sneezing and laughing due to a Valsalva type effect.

However, little data exists in the contemporary literature to describe the forces, which occur at rest and during routine activities. One common method for the recording the pressures experienced in the diseased state is during urodynamic (pressure-flow) assessment. The intra-vesical pressure can be calculated using a pressure- transducing catheter and is commonly measured in centimeters of water (cmH_2O). DeLancey [134] described that the pressure inside the bladder during standing with a relaxed bladder is on average $40cmH_2O$. It can be surmised that because the bladder is located within the pelvis, just superior to the pelvic floor connective tissue, that this pressure can equate to the hydrostatic load placed upon the pelvic floor. If the mean contact area of the female pelvic floor is taken as $94cm^2$ [134], Delancey calculated that the forces which act on the pelvic floor in the standing position, supine position, during coughing and straining were 37 Newtons (N), 19N, 129N and 92N, respectively [135]. This highlights the sudden and acute forces that are experienced during even routine activities of daily living.

1.6.1 Mechanical testing

Unlike muscle, the pelvic floor connective tissue is described as passive; it can transfer and resist force, however it is unable to generate it. The tensile properties of passive materials are determined by several factors, in terms of the pelvic floor connective tissue, the ECM is important. Here, the exact composition of the ECM, the spatial relationships and organization.

Biomechanics, is the application of mechanical testing to biological systems and

therefore, this term will be applied to biological tissue only, herein. There are two important distinctions to be made during mechanical testing:

1. **Structural characteristics** – the displacement of a material/tissue in response to load.
2. **Mechanical characteristics** – the stress (calculated as force / cross-sectional area) and strain (calculated as displacement relative to the initial length of a material/tissue) relationship.

Mechanical characteristics take into account the area of the material/tissue and therefore use force per unit area as a measurement. As such, the latter is perhaps a more accurate representation of the structure and composition of a tested sample.

1.6.1.1 Tensile testing

A variety of methods can be used to test the mechanical characteristics of a sample. The most commonly performed is the uniaxial tensile test. This relies on applying force to a sample along a single axis. A material/tissue is clamped between two grips; one of which is connected to a load cell. The sample is then elongated at a set rate to the point of failure.

The values for stress (N) and strain at corresponding time points can be plotted graphically to produce a stress-strain curve. Stress-strain curves are generally non-linear but commonly demonstrate a linear portion depending on the material properties. The initial phase of a stress-strain curve represents a material clamped between the grips in either a 'flaccid' or 'crimped' state, where the degree of passive elongation does not correspond with significant levels of stress. A linear portion follows this initial 'toe' phase, which is associated with a relatively elastic elongation of the tested sample. At the elastic limit, the sample becomes plastically deformed prior to failure. Schematic representation of a stress-strain curve is demonstrated in figure 1.6.1.

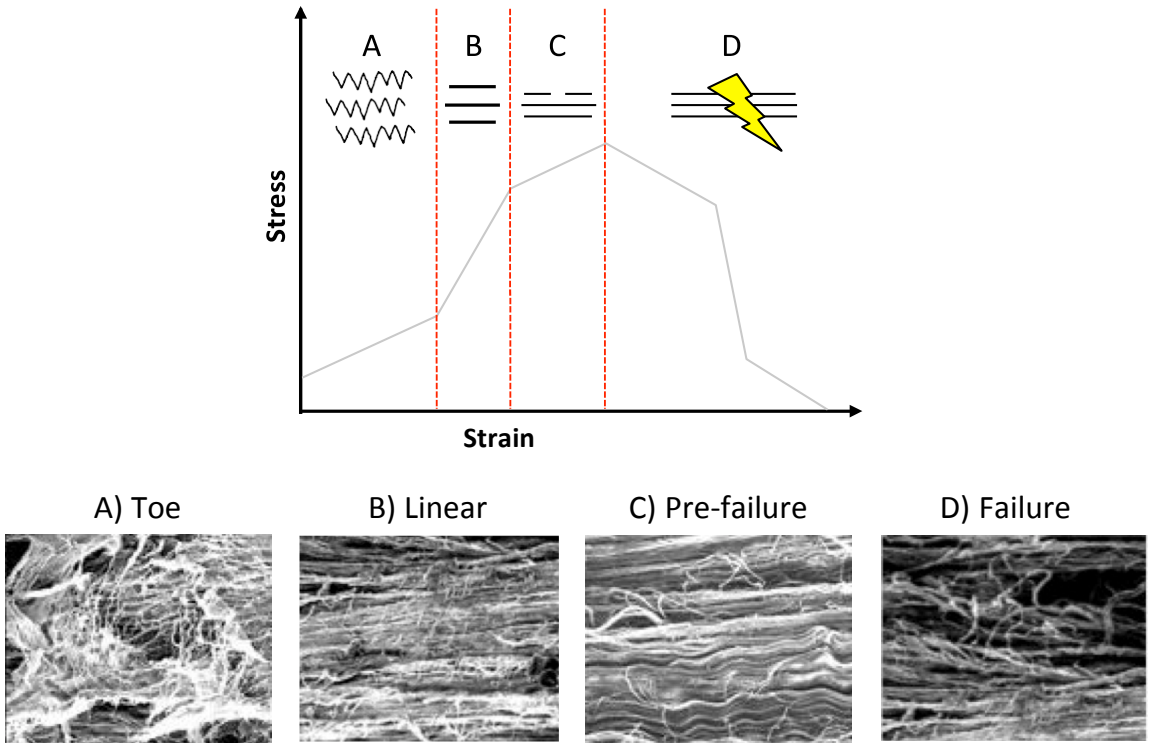


Figure 1.6.1. ***Representation of stress-strain curve for connective tissues that behave in a non-linear mechanical fashion***

Top – stress-strain curve of a typical collagen containing connective tissue, which exhibits an initial 'toe' phase (A) during elongation, followed by a linear region (B), at which point the Young's modulus can be calculated. A pre-failure (C) phase is the point at which the tissue/material undergoes irreversible plastic deformation and the maximal point of stress is taken as the ultimate tensile strength, following which the material fails (D). Bottom – representative images of tissue fibres during elongation to demonstrate crimping-uncrimping, followed by 'fraying' of the fibres and ultimate failure of the tissue.

Several values can be derived from the stress-strain curve, used to describe the mechanical properties of the material/tissue itself. The Young's modulus (YM) is calculated from the slope of the initial linear portion of the curve and represents the stiffness/elasticity of the sample. The ultimate tensile strength (UTS) represents the maximum peak of the curve; the point at which the maximal stress is reached prior to failure and is measured in N/mm^2 or Megapascals (MPa).

In order to accurately record the mechanical characteristics of a sample, the cross-sectional area must be calculated. This can also allow comparisons to be made with samples of differing size. The cross-sectional area can be calculated using contact or non-contact methods [136, 137]. Contact methods are commonly performed using a micrometer, which can lead to compression of the sample and therefore inaccurate

recording. Non-contact methods, such as laser micrometery are more accurate but less commonly performed.

1.6.1.2 Problems with tensile testing

The uniaxial test is a useful and rapid method for providing useful and representative data about a particular material/tissue. However, while the uniaxial test can be reliable for testing synthetic materials that have a uniform dimensional structure, biological tissues rarely behave in this way. Testing biomaterials along one axis may not be a representative test for a tissue that is designed to provide support in a multi- axial fashion. Furthermore, taking a sample of tissue and testing it *ex vivo* may not be representative of the function with which it behaves *in vivo*.

Axial coupling is another complicating factor; the mechanical properties of a material in one axis being dependent upon the force applied to the same material in another axis [138]. These factors argue in favour of multi-axial tensiometery, the protocols for which are increasingly complex and more difficult to standardize [138].

1.6.2 Biomechanical properties of connective tissues

Connective tissues are not completely elastic. Instead, they demonstrate viscoelastic behaviours, possessing features of both a viscous fluid and an elastic solid [139-141]. After loading, the stress-strain curve of connective tissues does not return along the same course during relaxation. This property is termed hysteresis (Figure 1.6.2A). Viscoelastic tissues demonstrate other behaviors, which result as a consequence of this dissipation of energy. 'Creep' represents elongation of a material over time, while being subjected to a constant load. 'Stress-relaxation' refers to the reduction in stress that occurs when a material is subjected to a constant elongation. Akin to this is a property, whereby following repeated cycles of constant strain, a reduction in stress occurs with each subsequent cycle. The result of this is a rightward shift in the loop on a stress-strain curve (figure 1.6.3B). This behavior is termed the Mullins effect and represents a material that becomes easier to stretch with

successive stretches [142, 143]. However, after each successive cycle, the degree of hysteresis reduces and the loading/relaxation curves become closer together. For this

reason, a period of 'warming up' can help to avoid muscle strain, associated with excessive stress during initial staining.

Connective tissues contain elastin, collagen and proteoglycans that facilitate the trapping of water molecules. The interplay between these substances is responsible for the mechanical behaviors that these tissues demonstrate.

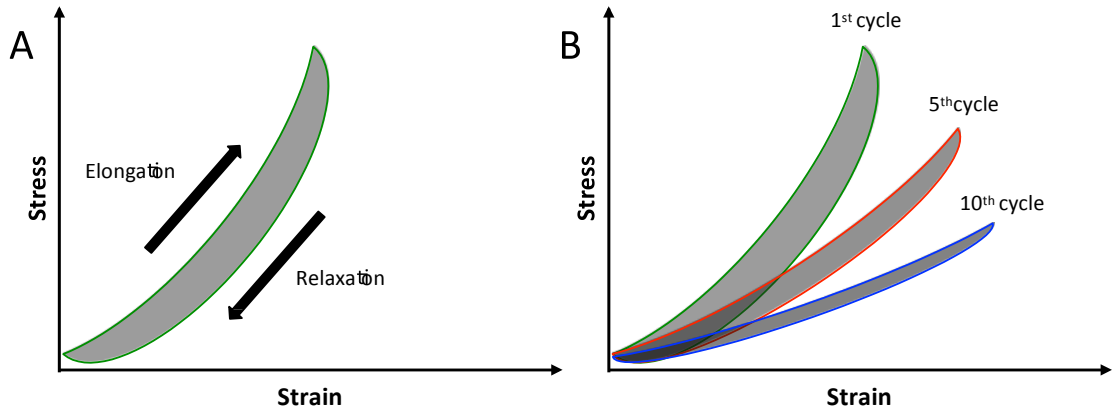


Figure 1.6.2. ***Viscoelasticity of tissues***

A) Stress-strain curve to demonstrate hysteresis. The tissue/material undergoes cycles of elongation and relaxation; the curve follows two separate paths, indicating dissipation of energy. B) The rightward shift of hysteresis curves that occur following cycles of successive strain.

1.6.3 Review of the biomechanical properties of pelvic floortissues

The majority of studies that assess the biomechanical properties of pelvic floor tissues in humans use vaginal tissues for comparison between patients with POP ± SUI rather than the pelvic ligaments and musculature. These tissues are primarily obtained during routine gynaecological surgery for the treatment of POP or SUI. Other tissues are obtained from cadaveric tissue. The majority of studies use uniaxial testing for comparison, describing the Young's modulus and ultimate tensile strength values. Other methods for comparison are not described herein.

There is considerable variability in the exact tissues that are tested and the YM/UTS results that are obtained as a result (Table 1.6). In general, all studies demonstrate a difference between the values for both Young's modulus and ultimate tensile strength for vaginal tissue associated with POP *versus* control tissue. Of studies that

demonstrate the YM for POP versus non-POP tissues [144-146], both Martins and Lei *et al* [144] demonstrate lower values for non-POP tissues as compared to POP In another study, Jean-Charles *et al* [147] used mathematical modeling to show that POP tissues have a higher YM. Zimmern [145], however showed that POP tissues had a lower YM, while using a different hydration protocol than the other studies.

For UTS, three studies demonstrated higher values for tissues taken from women with POP compared to controls [145, 146, 148]. Meanwhile, Lei *et al* [144] showed that tissues taken from control patients had a higher UTS; those taken from pre-menopausal patients being both stronger and less stiff than those from post-menopausal women.

In summary, the data relating to the biomechanical properties of pelvic floor tissues in humans is relatively sparse. The generally accepted view is that tissues affected by POP and SUI are generally stiffer and weaker. However, the published evidence is conflicting. Several possible explanations for these findings exist. There are many confounding variables in these patient cohorts, aside from simply the presence or absence of POP/SUI. We have described that menopausal status [149], hormone replacement therapies [150], weight, parity, mode of delivery and race are confounding factors and these are not controlled for in these studies. Furthermore, the exact method of tissue sampling and testing is important, as anisotropy exists in these tissues. Clearly, the biomechanical tissues are affected during storage and this can have implications on the results of cadaveric tissue. Other factors, such as storage temperature, hydration state [151] and thawing conditions [144] can have an impact upon the mechanical properties.

Despite these explanations, it is difficult to reconcile those studies that demonstrate stronger and stiffer tissues that are taken from patients with POP. One possible explanation is that these tissues are undergoing constant remodeling; a process that is associated with a reduction in the collagen I/III ratio, despite a 'normal' total collagen content [106]. Furthermore, no study assessed the vital role that the pelvic ligaments and other connective tissues play in the clinical findings of POP and these tissues may undertake a compensatory role. However, the metabolic changes seen in the vaginal

tissues are likely to be part of a systemic problem and other connective tissues may be affected in addition to the vaginal tissues.

Table 1.6. *Published literature that relates to uniaxial biomechanical testing of vaginal tissues*

Study	Tissue	Comparators	YM	UTS	Conclusions
Goh 2002 **[149]**	Anterior vaginal wall Living donors	Pre-menopausal POP Vs Post-menopausal POP	11.5 14.35	- -	Significantly greater YM in post-menopausal tissue.
Lei 2007 **[144]**	Anterior vaginal wall Living donors	Pre-menopausal No POP (n=14) Pre-menopausal POP (n=9) Post-menopausal No POP (n=14) Post-menopausal POP (n=9)	6.65 9.45 10.26 12.10	0.79 0.60 0.42 0.27	Pre-menopausal tissues significantly lower YM and greater UTS than post-menopausal. POP stiffer and weaker than no POP.
Rubod 2008 **[148]**]	Anterior vaginal wall Living donors (POP) Cadaver (no POP)	Post-menopausal No POP (n=5) Post-menopausal POP (n=5)	- -	1.398 3.82	Significantly greater UTS for POP. However, issues normalizing cross-sectional area.
Zimmern 2009 [145]	Anterior vaginal wall Living donors (POP) Cadaver (no POP)	No POP (n=3) POP (n=23)	10.2 8.4	1.4 2.1	POP tissues more elastic and stronger than No POP.
Jean-Charles 2010 **[147]**	Full-thickness vaginal wall	No POP (n=10) POP (n=19)	Assessed using mathematical modeling to compare YM at both low and high deformation.		POP significantly greater YM (stiffer) than controls.
Martins 2013 **[146]**	Anterior and posterior vaginal wall Living donors (POP) Cadaver (no POP)	No POP (n=15) Anterior Posterior POP (n=40) Anterior Posterior	 6.9 10.5 13.1 9.5	 2.6 3.5 5.3 3.2	POP higher YM and UTS (stiffer and stronger).
Ranges		No POP POP	6.65-10.26 8.40-14.35	0.42-2.60 0.27-5.30	

POP = Pelvic organ prolapse

YM = Young's modulus

UTS = Ultimate tensile strength

1.7 Treatments for POP/SUI

In the UK, the costs related to the management of urinary incontinence occupies 0.3% of the entire National Health Service (NHS) budget [152], while in the US, the annual direct costs of SUI was estimated at $13.12bn in 1995 [25]. In England alone, prolapse surgery represented an estimated at 28,959 hospital admissions in 2005, accruing a total cost of €81.03 million and similar findings are observed in other European nations including Germany and France [55]. In 1997, the cost of POP surgery in the US was estimated to be over $1bn, for a total of 226,000 procedures [54].

Patients with POP or SUI are initially managed by identifying and adjusting any modifiable risk factors, such as obesity, chronic cough and constipation. Medical therapies are available, however the majority of patients ultimately undergo surgery for the treatment of their condition.

In the last 20 years, two major advances in the field of reconstructive urology have come to the fore: The availability of biomaterials and the introduction of minimally invasive techniques. One such advancement is the use of mesh materials for the treatment of SUI and more recently for POP. Mesh tape surgery as the tension-free transvaginal tape (TVT) was developed by Petros and Ulmsten [153] in the 1990's based upon the integral theory of urinary continence [62]. The aim was to simplify the pubo-vaginal sling procedure, which uses autologous fascia and was popularized by Edward McGuire in the 1970's [154]. Mid-urethral tape (MUT) surgery using polypropylene mesh (PPL) gained popularity following large scale randomized controlled trial data demonstrating rapid recovery and comparable success rates to colposuspension [155]. The technique has evolved rapidly and a plethora of procedures and 'kits' have subsequently emerged, including the transobturator tape (TOT) and single-incision or *mini-sling* kits in an attempt to overcome the blind passage of trocars through the retropubic space, which risks bladder and bowel injury. Irrespective of the route used, in experienced hands the success rates of mesh surgery for incontinence are high in the medium term [156] and are similar to those for pubo-vaginal slings using autologous rectus fascia [157] and avoids the need for a further lower abdominal incision, which has implications on cosmesis, pain and infection. The artificial urinary sphincter (AUS) device has been used for almost 30 years, with very little change to the

underlying design. It is successfully used in the treatment of male stress urinary incontinence, such as that which occurs following radical prostatectomy or in patients with neurological disease. It is less commonly used in women with stress urinary incontinence, where it's role is limited to the treatment of intrinsic sphincter deficiency, a more severe form of SUI. The outcomes of the AUS are therefore not discussed further, as very little randomized trial data exists in the literature that directly compares AUS with conventional anti-incontinence surgery for the treatment of uncomplicated SUI.

Following the early data in support of PPL mesh use for SUI, mesh has been increasingly used in prolapse surgery and its use to reinforce the repair of anterior compartment prolapse is associated with cure rates of 91.8% compared to 71.2% for standard colporrhaphy without mesh [158].

We discuss the treatment strategies employed by clinicians for the treatment of both POP and SUI, including a review of the outcomes of contemporary biomaterial surgical techniques *versus* traditional surgery.

1.7.1 Conservative treatment of pelvic floor disorders

With the increasing awareness of the FDA reports on the safety of mesh devices for use, particularly in prolapse surgery, a stepwise treatment protocol is important in the management of patients with pelvic floor disorders.

Correction of identifiable risk factors, such as smoking and obesity may be of some benefit to patients. Patients with morbid obesity are at a significantly greater chance of developing prolapse compared to age and parity matched controls [159]. The International Continence Society recommendations suggest that patients with prolapse should undergo supervised pelvic floor muscle training [2]. Pelvic floor muscle training was originally introduced by Kegel in the 1940's [160] and aims to improve contraction and tone of the levator ani muscle group. Studies have demonstrated that pelvic floor training is associated with significant improvements on scores of patient reported outcome measures [161], while other data shows that pelvic floor training slows the progression of anterior prolapse in elderly women [162].

Morbidly obese patients are at a 5 times greater risk of developing incontinence than patients with a body mass index of 25. Furthermore, weight loss of 10% in these patients confers a 50% reduction in urinary leakage [163].

1.7.2 Mechanical devices for prolapse

Pessaries have been used to treat prolapse for centuries. These devices work by providing rigid mechanical support inside the vagina to prevent the herniation of pelvic organs. Such treatments are regarded as safe and patient satisfaction is generally high and it is estimated that up to two thirds of patients with prolapse would opt for pessary treatment over primary surgery [164]. Despite this, data relating to the long-term outcomes of pessary use in the literature is lacking, however, 77% of members of the American Urogynecologic Society report using pessary treatments as first-line therapy for prolapse [165]. These devices are easy to insert, should be comfortable for the patient and should effectively reduce the prolapse. Wu et al report successful fitting of pessaries in 74% of patients [166], with most failures or discontinuation occurring within one month of initial fitting [167]. Many types of pessary are available for treatment and can generally be classified into supportive (ring) or space-filling (Donut or Gellhorn).

1.7.3 Medical treatment of pelvic floor disorders

There has been some interest in the use of several pharmacotherapies for the treatment of stress urinary incontinence. The group of tricyclic antidepressants including imipramine, are thought to have their effect by reducing the contractility of the bladder and improve the resistance of the urethra to urinary flow. Duloxetine, a dual serotonin/noradrenaline re-uptake inhibitor, also increases the urethral resistance to micturition by having it's effect on the alpha-adrenoreceptors and gamma-Aminobutyric acid receptors of the urethral sphincter mechanism. Both of these drug therapies demonstrate poor results measured by pad testing patients with SUI [168, 169].

It is well documented that the incidence of pelvic floor disorders increases following the menopause, which is likely due to the reduction in circulating and local levels of oestradiol. It is thought that the role of oestradiol in the lower genitourinary tract is

to increase the sensitivity of smooth muscle receptors of the urethra and to also reverse the atrophic vaginitis associated with urethral resistance [170]. While studies have shown an increase in total collagen and reduction in the concentration of matrix metalloproteinases responsible for collagen degradation in subjects treated with topical oestradiol for 6 weeks in a randomized trial [171], others have suggested that the matrix component differences encountered are a result of the prolapse itself, rather than being dependent upon the serum oestradiol concentration [172]. Other data has demonstrated a significant reduction in MMP-1 from vaginal fibroblasts due to increased fragmentation of these collagenases resulting from treatment with oestradiol +/- progesterone [130]. Furthermore, MMP-13, responsible for the activation of other metalloproteinases has been demonstrated to be suppressed with hormone treatment, that also restores the pelvic floor mechanical properties in ovariectomized rats as compared with non-treated subjects [150]. Clinical trials have demonstrated the benefits of an oestradiol releasing vaginal ring in patients with atrophic vaginitis without any significant adverse events [173].

At present however, the results of systematic reviews conclude that there is no convincing evidence base to support the widespread use of either systemic or topical oestradiol for the treatment of SUI or POP in isolation [174] and that caution should be exercised due to the risk of endometrial or breast cancer.

1.7.4 Biomaterials for SUI and POP

The traditional surgical treatments of both SUI and POP rely upon the plication or anchorage of existing tissues using sutures. This constitutes what is known as a native tissue repair (NTR). As either degradable or non-degradable sutures are used, the integrity of a repair depends upon scar tissue formation; a process, which involves collagen deposition and is associated with a significantly lower proportion of elastin synthesis. The strength of the repair therefore relies upon the strength of the deposited unorganized collagen. However, these repaired tissues are inherently weaker than healthy connective tissues due to the lack of aligned collagen fibres, which occur along the longitudinal axis of stress.

Repair using biomaterials is a process, which aims to overcome this problem. A biomaterial is defined by the National Institutes of Health (NIH) as "any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as part of a system which treats, augments, or replaces any tissue, organ, or function of the body." [175].

1.7.4.1 Injectable materials for SUI

While surgical tapes or slings and the artificial urinary sphincter are used effectively to treat this condition, not all patients may be suitable. Urethral injection therapies were designed to effectively augment the function of the urethral sphincter mechanism in patients with ISD. By using a minimally invasive injection technique, this treatment can be potentially useful in those who are either too unfit or decline invasive surgical treatment of their condition (Figure 1.7.1).

Urethral bulking agents have become more popular over the last five decades in response to improved materials design, the minimally invasive fashion with which treatments can be administered and the relatively low morbidity associated with injections [176].

In the 1990s, a plethora of synthetic bulking agents emerged, including Macroplastique® ((silicone particles) Westborough, MA, USA), Coaptite® ((calcium hydroxylapatite) Bioform Medical inc, San Mateo, CA, USA), Durasphere® ((carbon-coated zirconium beads) Advanced UroScience inc, St Paul, MN, USA) and more recently Bulkamid® ((polyacrylamide hydrogel) Contura, Soeborg, Denmark). Each of these synthetic agents contain microspheres suspended in a matrix and the incidence of migration is reduced by using larger diameter spheres (>100μm). As compared with biological agents, whose degradation is unpredictable, synthetic injectables are more durable and offer potentially tunable mechanical properties.

1.7.4.1.1 Outcomes

Using autologous fat injections compared to placebo, Lee *et al* [177] demonstrated no significant improvements in continence rates 3 months following injection (22% versus 21% respectively), while the complications were significantly greater in the treatment group (32% versus 11% respectively), including one death from a fat embolism. Contigen™ has been demonstrated to result in 66% subjective continence rates when

injected at the level of the mid-urethra and increases the MUCP in patients with ISD [178]. Meanwhile, no significant differences in pad weight testing have been demonstrated for Contigen™ versus Macroplastique®, while the latter does demonstrate better subjective cure rates [179]. Macroplastique® has, however been demonstrated to result in significantly poorer objective outcomes than Permacol™ after 6 weeks, while the effects of Permacol™ are sustained beyond 6 months (41% versus 60% dry pad test respectively for Macroplastique® *versus* Permacol™) [180].

Of the newer synthetic agents, Anderson demonstrated significant improvements in both objective cure rates (pad testing) and subjective cure (Stamey grade) beyond 2 years of follow-up for Durasphere® over Contigen™ in those with ISD (VLPP <90cmH_2O) [181]. Coaptite® has been shown to result in significantly better long- term improvements in Stamey grade than Contigen™ and also requires significantly fewer injections over time (38% of patients only required one injection over 12 months for Coaptite® versus 26% for Contigen™) [182]. Similarly, Durasphere® resulted in significant improvements in Stamey grade over 12 months than Contigen™ (80% versus 69% respectively) and required less material [183]. Despite this, the rates of urinary retention in the immediate setting were significantly greater for Durasphere® over Contigen™ (16.9% versus 3.4% respectively) and similar findings have been demonstrated for a discontinued agent, Zuidex™ ((hyaluronic acid with dextranomer) Q-Med AB, Uppsala, Sweden) as compared to Contigen™ [184]. Sokol *et al* [185] demonstrated no significant objective improvements in patients injected with Bulkamid® over Contigen™, however 77.1% and 70% respectively considered themselves cured or improved. There were no significant differences between these two agents and few overall adverse events were reported in this multicentre randomized study.

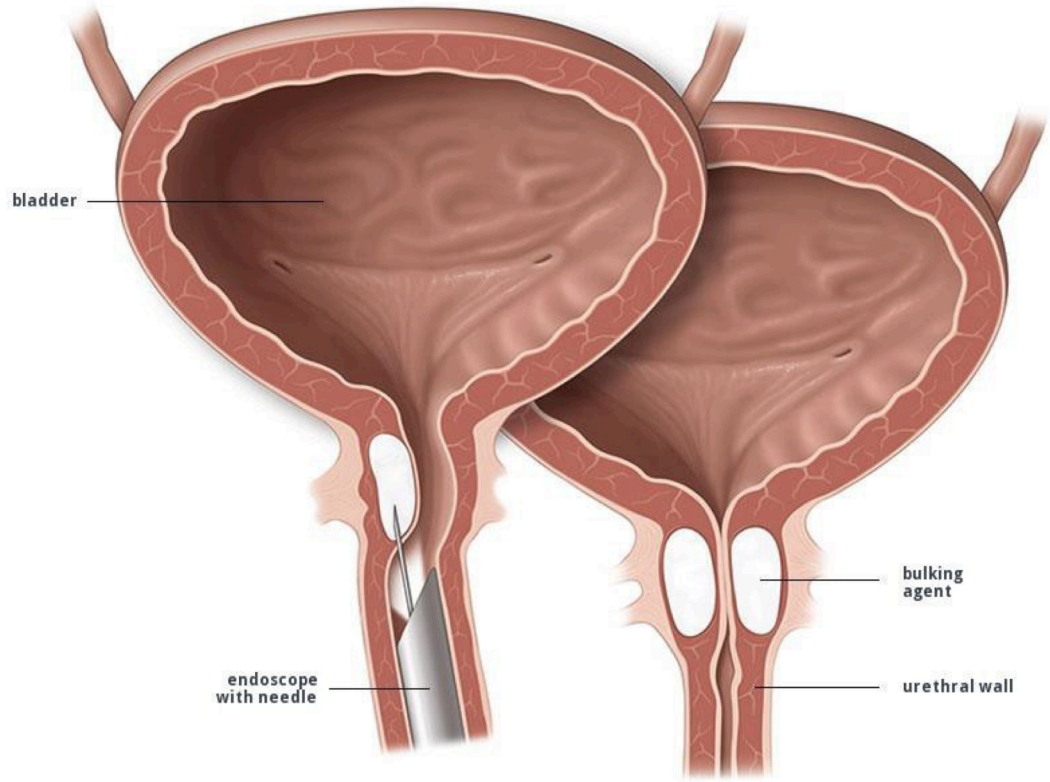


Figure 1.7.1. ***Technique for the injection of urethral bulking agents***

Cystoscopic guidance is used to direct the intramural injection of agents using a needle. Reproduced from EAU patient information leaflets.

1.7.4.2 Synthetic mesh for SUI/POP

Synthetic surgical mesh is typically composed of a non-biodegradable, thermoplastic polymer. 4 different types of synthetic mesh exist, depending upon their pore size and filamentous structure (Table 1.7.1) [186]. In this context, macroporous mesh is defined as a pore size in excess of 75µm, while microporous mesh contains a pore size below 10µm. The filamentous structure refers to each individual fibre. Mono- filament meshes are composed of a single polymer fibre that is woven into the mesh structure, while a multi-filamentous mesh comprises bundles of fibrils, which form each filament that is then woven into the mesh structure (Figure 1.7.2).

Type I mesh is most commonly used in routine clinical practice, with a variety of applications in abdominal wall defect repair, for anti-incontinence surgery and prolapse repair. In this context, the mesh material usually contains a pore size of 150µm, with a mono-filamentous structure using polypropylene (PPL).

Table 1.7.1. *Classification of mesh by type*

Type	Example	Pore size	Filament	Image
I	Polypropylene Marlex	Macroporous >75µm	Mono-filamentous	A/B
II	Expanded polytetrafluoroethane (PTFE)	Microporous <10µm	Mono and multi-filamentous	C
III	Polyethylene terephthalate (PET) Polyglycolic acid (PGA)	Macroporous	Multi-filamentous	D
IV	Polyatex	Non-porous	Mono-filamentous	E

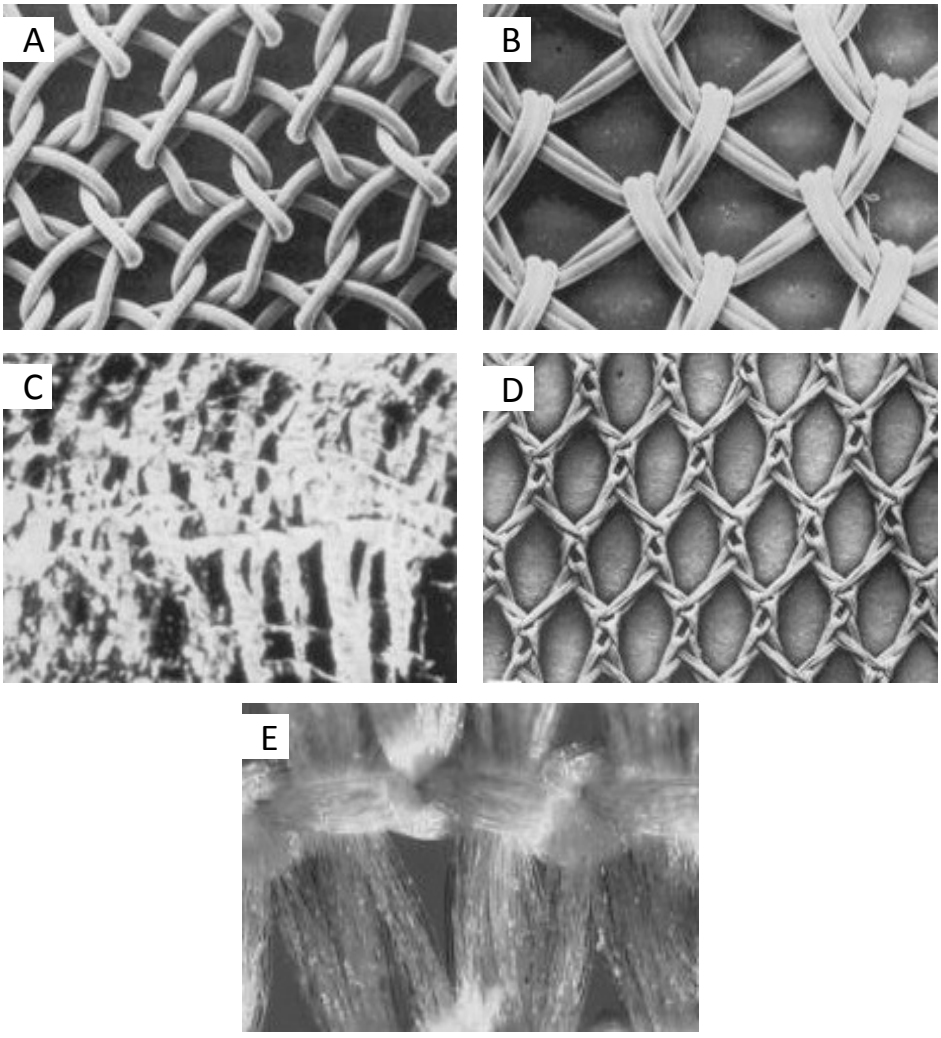


Figure 1.7.2. ***Microscopic appearances of surgical mesh***

A) Marlex (CR Bard, Cranston RI). Monofilamentous polypropylene (Type I mesh).

B) Prolene (Ethicon, Somerville, NJ). Monofilamentous polypropylene (Type I mesh).

C) Gore-Tex (WL Gore, Flagstaff, AZ). Monofilamentous ePTFE (Type II mesh).

D) Mersilene (Ethicon, Somerville, NJ). Multifilament PET (Type III mesh).

E) Surgipro (Boston Scientific, Natick, MA). Multifilamentous polypropylene (Type IV mesh).

Reproduced with permission from Nelson, E et al. Influence of mesh structure on surgical healing in abdominal wall hernia repair. 10th World Biomaterials Congress. doi: 10.3389/conf.FBIOE.2016.01.01142

1.7.4.3 Biological grafts used for SUI/POP

The biological materials that are used for reconstructive surgical procedures can be classified as either autografts, allografts or xenografts (Table 1.7.2). In the context of pelvic floor reconstruction, autografts are harvested as fascia from either the rectus sheath of the abdomen or fascia lata of the thigh. Autologous grafts are usually harvested at the time of reconstructive surgery, however they depend upon the tissue quality and availability, which can become a problem following abdominal surgery. Furthermore, several complications can occur at the site of harvest, including wound infection, chronic pain and hernia formation that results due to a deficiency of the fascial layer of the abdominal wall.

To avoid these local complications associated with autologous fascia harvest, allografts can be used. These tissues are taken from cadaveric donor tissue banks and comprise dermis or cadaveric fascia. Tissues are decellularised and processed to render them non-immunogenic. One concern with this is that these grafts are often taken from elderly patients and therefore age related structural changes are associated with unpredictable tensile properties and ECM composition [187]. Furthermore, the processing techniques that allografts undergo, such as freeze drying can further affect the biomechanical properties of these tissues; ice crystals form which weaken the collagen structure in the ECM [188]. The rare but significant risk of disease transmission exists with allografts taken from donor tissue banks, in particular prion diseases and human immunodeficiency virus (HIV). For this reason with all human tissue banks in the UK, robust screening processes are performed and there is yet to be a case of disease transmission following allograft tissue surgery.

Xenografts, such as porcine dermis, porcine small intestinal submucosa (SIS) and bovine pericardium were introduced to overcome the problems associated with cadaveric tissue. These tissues undergo decellularisation and processing to remove the cellular components that are involved in the immune response. Chemical cross-linking of xenografts is a recent advancement in an attempt to prevent enzymatic degradation following implantation.

The decellularisation, processing and sterilization methods that allograft and

xenograft materials undergo are associated with negative outcomes on the mechanical properties. While newer generation grafts undergo less robust methods of processing in order to preserve the ECM components, the concern is that DNA is still found in allografts [189] and xenografts [190].

Table 1.7.2. *Biological grafts used for SUI/POP surgery*

Graft	Generic name	Modification	Sterilization	Collagen content	Elastin content	Clinical usage
Autologous fascia	Nil	Nil	Nil	High	Moderate	+++
Cross-linked porcine dermis	Pelvicol Pelvilace	Cross-linked with hexamethylene-diisocyanate (HMDI)	Gamma-irradation	High	High	++
Porcine small intestinal submucosa	Surgisis	Nil	Ethylene oxide	Moderate	Low	+
	Fortagen	Cross-linked with carbodimide	Gamma-irradiation	Moderate	Low	-
Cadaveric dermis	Tutoplast	Freeze dried Solvent dehydration	Gamma-irradiation	Moderate	High	-
Cadaveric fascia	Tutoplast	Freeze dried Solvent dehydration	Gamma-irradiation	Moderate	High	-
Bovine pericardium	Veritas	Nil	Gamma-irradiation	Moderate	Moderate	-

+++ Most commonly utilized biological graft

- Infrequently used

1.7.5 Surgical outcomes in SUI/POP

Comparing the outcomes between different studies for SUI/POP surgery is difficult, as different assessment methods, surgical approaches, outcome measures and follow-up protocols are used by investigators.

In this section, studies are described that satisfy the following criteria:

1. Clearly evaluate separate biomaterials
2. Define objective outcome measures, e.g pad weight testing, clinical examination and not just subjective outcomes.
3. Reported complications of procedure/material.
4. Sufficient follow-up data (>1 year).
5. Randomized control trial and meta analysis data only, case series are not included.

1.7.5.1 SUI surgery – Native tissue repair (NTR)

Retropubic suspension procedures are used in patients with urethral hypermobility. The original intention with this approach was to restore the urethra to the intra-abdominal position where it would receive equal pressure transmission as the bladder, therefore closing effectively during stress.

Four variations of open retropubic susension exist that differ in terms of the degree of elevation and support that they confer upon the urethra; the Marshall- Marchetti-Krantz (MMK) procedure, the Burch colposuspension (BC), vaginal- obturator shelf (VOS) procedure and the paravaginal repair.

1.7.5.1.1 The Marshall-Marchetti-Krantz (MMK) procedure

The authors first described this retropubic technique in 1949 [191]. Three individual suture bites are taken of the paraurethral fascia and the anterior vaginal wall (excluding mucosa) and are anchored to a corresponding cartilagenous portion of the symphysis pubis.

Mainprize and Drutz [192] reviewed the outcomes of 2712 cases, demonstrating improvement rates of 91% based on subjective criteria. Success rates of primary versus repeat procedures were 92.1% versus 84.5% respectively. However, osteitis

pubis, a potentially devastating complication, occurred in 2.5% of patients. The MMK procedure is no longer recommended for the treatment of SUI by the 4th International Consultation of Incontinence [2].

1.7.5.1.2 Burch colposuspension (BC)

The Burch colposuspension aims to elevate the paravesical tissues towards the iliopectineal line of the pelvic sidewall (figure 1.7.3).

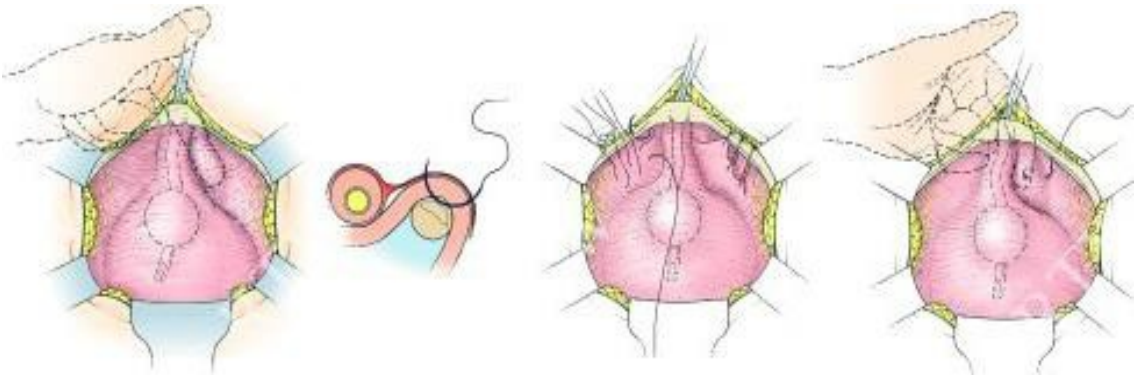


Figure 1.7.3. ***The Burch colposuspension***
Attaching the paravaginal tissues to Cooper's ligament. Reproduced with permission from Turner-Warwick T and Chapple C. Functional reconstruction of the female genito-urinary tract. Blackwell 2002.

Meta analyses have demonstrated objective continence rates with the Burch of 84% in over 1700 patients with follow-up between 1 and 60 months [193]. Lapitan [194] reviewed 53 trials including 5244 patients and demonstrated overall success rates of 68.9% to 88% and after 5 years, approximately 70% of patients can expect to be dry. Meanwhile, whilst previous surgery can prevent adequate suspension, Maher *et al*
[195] have shown subjective cure rates of 89% with repeated colposuspension after 9 months of follow-up.

1.7.5.1.3 Paravaginal repair

As compared to the BC, the arcus tendineus ('white line' of the pelvis) is used for attachment of the paravaginal tissues. Richardson *et al* [79] re-popularized an abdominal approach of White's [196] original method from 1909. In Richardson's description, the bladder and urethra are not mobilised from their vaginal attachments.

1.7.5.1.4 Vaginal-obturator shelf repair (VOS repair)

Turner-Warwick described this variant of the paravaginal repair, which aims to prevent the urethral tethering that can occur following other retropubic suspension procedures. The vaginal wall and endopelvic fascia are approximated to the internal obturator muscle.

Unfortunately, the lack of data available for this technique has prevented definitive conclusions to be drawn to support its widespread use. German *et al* [197] randomized 50 patients to receive the vaginal-obturator shelf procedure or needle suspension. Overall, 70% of patients were continent following VOS repair, versus 57% for those undergoing needle suspension.

1.7.5.1.5 Laparoscopic retropubic suspension

Laparoscopic variations of the BC, MMK and paravaginal repairs have been performed using a trans-peritoneal or extra-peritoneal approach. The advantages of a laparoscopic approach over the open technique is improved intraoperative visualization, reduced post-operative pain and hospital length of stay. Despite this, a steep surgical learning curve, increased operating times and higher costs makes this technique unfeasible at some institutions.

Two large scale RCTs comparing the outcomes of laparoscopic versus open colposuspension have demonstrated similar success rates. Carey *et al* [198] randomized 200 patients and described similar overall objective cure rates (USI) at 6 months (75%) and subjective cure rates at 2 years of follow-up (66%). The operating time for laparoscopic colposuspension was double that of open colposuspension. These findings were supported by Kitchener *et al* [199], who reported on the results of the COLPO trial. Objective cure rates at 24 months (negative pad testing) were 79% for laparoscopic and 70% for open colposuspension. Intraoperative complications were low in both arms, with more bladder and bowel injuries in the laparoscopicgroup, whereas median operating time was similar between the two groups (65 and 51 minutes respectively).

1.7.5.2 SUI surgery – biomaterial sling/tape procedures

In this context, the term 'sling' relates to the use of biological tissue, whereas 'tape' refers to synthetic materials. The pubovaginal sling (PVS) was introduced over 100 years ago. A strip of fascia is placed at the bladder neck/proximal urethra, in an effort to correct urethral hypermobility. Using this technique, Blaivas *et al* [66] were able to obtain 94% cure rates at a follow-up of 18 months. In the 1990's Petros and Ulmsten's integral theory [62] led to the development of procedures such as the tension free vaginal tape that seek to provide support to the mid-urethra, which facilitates the effective kinking during increases in intra-abdominal pressure.

1.7.5.2.1 Pubovaginal sling (PVS)

The original use of fascial slings was reported by Aldridge in 1942. McGuire reintroduced the PVS in women with failed retropubic procedures [154]. Currently, indications for the pubovaginal sling include SUI associated with hypermobility and intrinsic sphincter deficiency or SUI with other concomitant defects.

Figure 1.7.4 highlights the usual donor sites for harvest. Allografts and xenografts are associated with unpredictable degradation and the theoretical risk of disease transmission [200, 201], whilst synthetic materials are associated with a high risk of infection and erosion, due to the positioning of the graft at the bladder neck, and hence are no longer used.

The full-length vaginal-suprapubic fascial sling is placed at the bladder neck/ proximal urethra and can be inserted via a retropubic approach or a primary vaginal approach with suprapubic abdominal wall suture-anchorage. The 'sling-on-a-string' procedure (figure 1.7.5) was developed because the fascial strip from harvesting the rectus fascia is relatively short. Although initially suture-dependent, the strip becomes incorporated during healing.

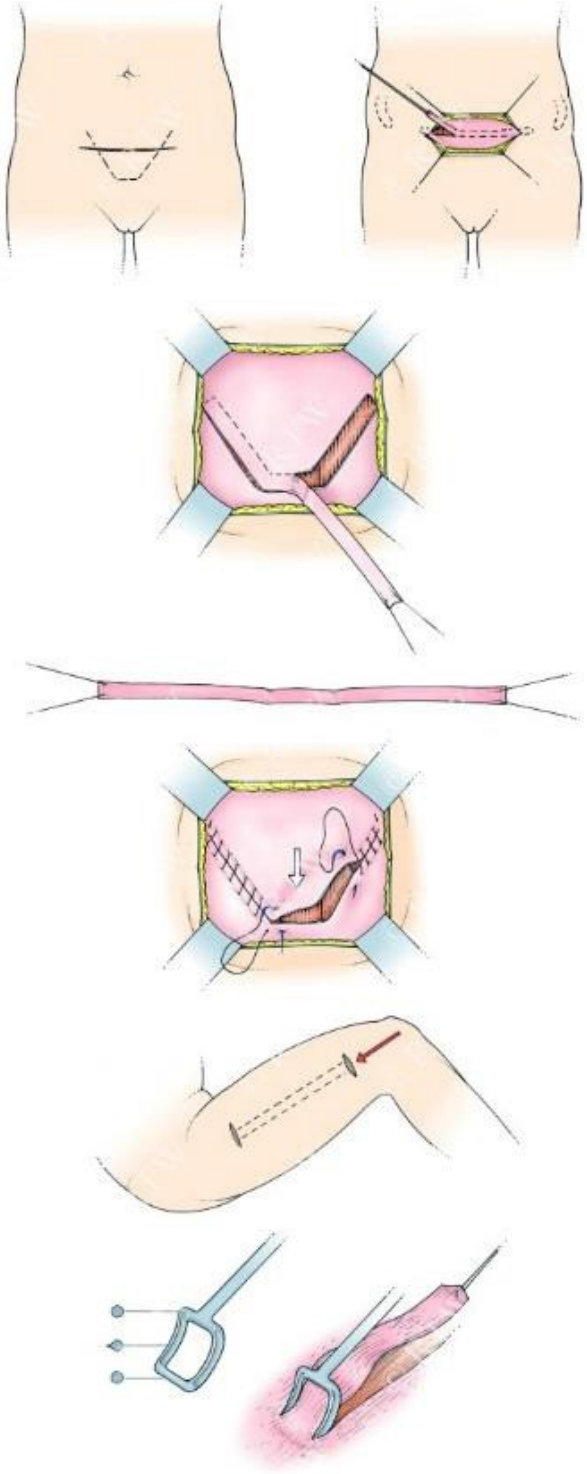


Figure 1.7.4. ***Donor site options for fascial strips – rectus fascia and fascia lata of the thigh.***

Reproduced with permission from Turner-Warwick T and Chapple C. Functional reconstruction of the female genito-urinary tract. Blackwell 2002.

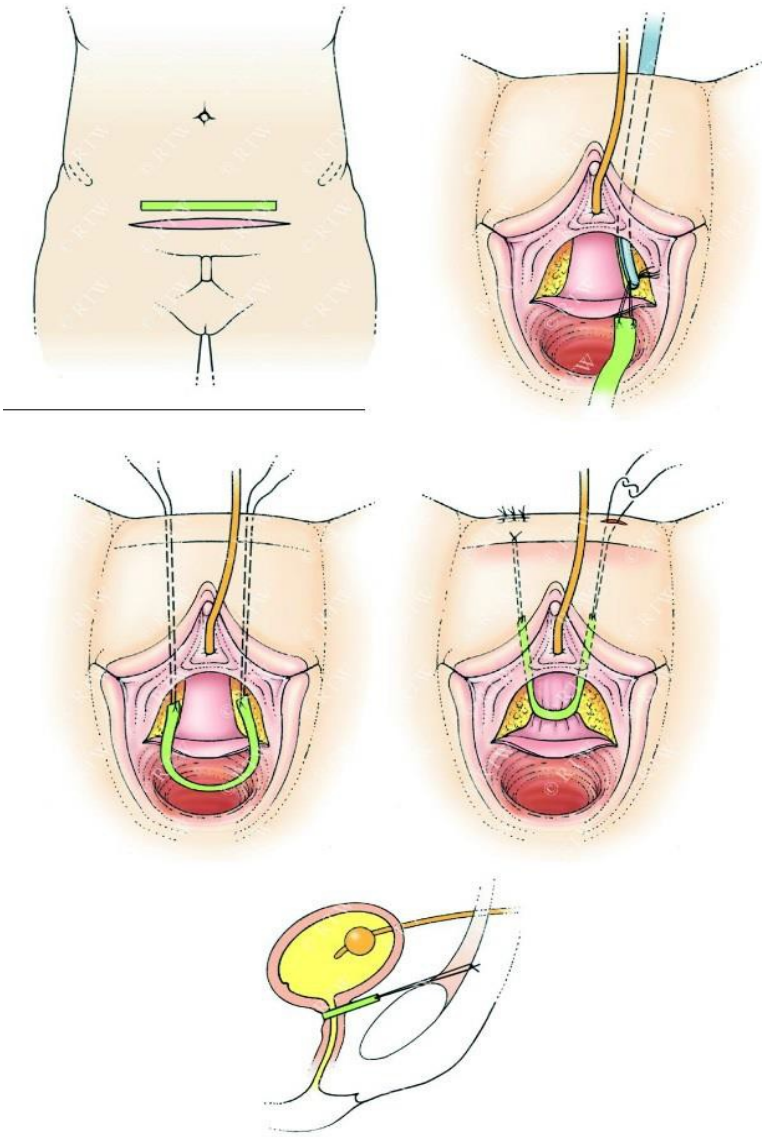


Figure 1.7.5. ***Autologous fascia sling-on-a-string procedure***
Reproduced with permission from Turner-Warwick T and Chapple C. Functional reconstruction of the female genito-urinary tract. Blackwell 2002.

1.7.5.2.2 Mid-urethral tape (MUT)

The integral theory highlighted the importance of the mid-urethral mechanism involved in continence and led to the introduction of the tension-free vaginal tape (TVT) in the 1990s [153, 202]. This minimally invasive approach was thought to work by providing a physiological 'backboard' by fixation of the middle part of the urethra to the pubic bone, via the pubourethral ligaments.

The TVT procedure is illustrated in figure 1.7.6. A synthetic polypropylene monofilament mesh, housed in a plastic sheath to aid retropubic passage, is placed

using trocars, typically performed in the bottom-to-up direction.

Since the inception of the original TVT procedure, a plethora of commercially available kits have been developed with modifications on the original approach. One such modification is using the top-to-bottom approach, which was developed to allow better control of the trocar through the retroperitoneal space, reducing the likelihood of vascular and bowel injury.

The transobturator tape approach (TOT) was then introduced by Delorme [203]. Although originally described as an 'outside-in' approach, subsequently De Leval [204] modified it to having an 'inside-out' approach (TVT-O). The transobturator approach avoids the passage through the retropubic space, thereby avoiding injury to bowel and bladder. The single incision sling was developed to further reduce the morbidity of the procedure, with the tape guided through a single incision in the anterior vaginal wall with trochars piercing the obturator fascia. The surgical outcomes for each are discussed in the following section.

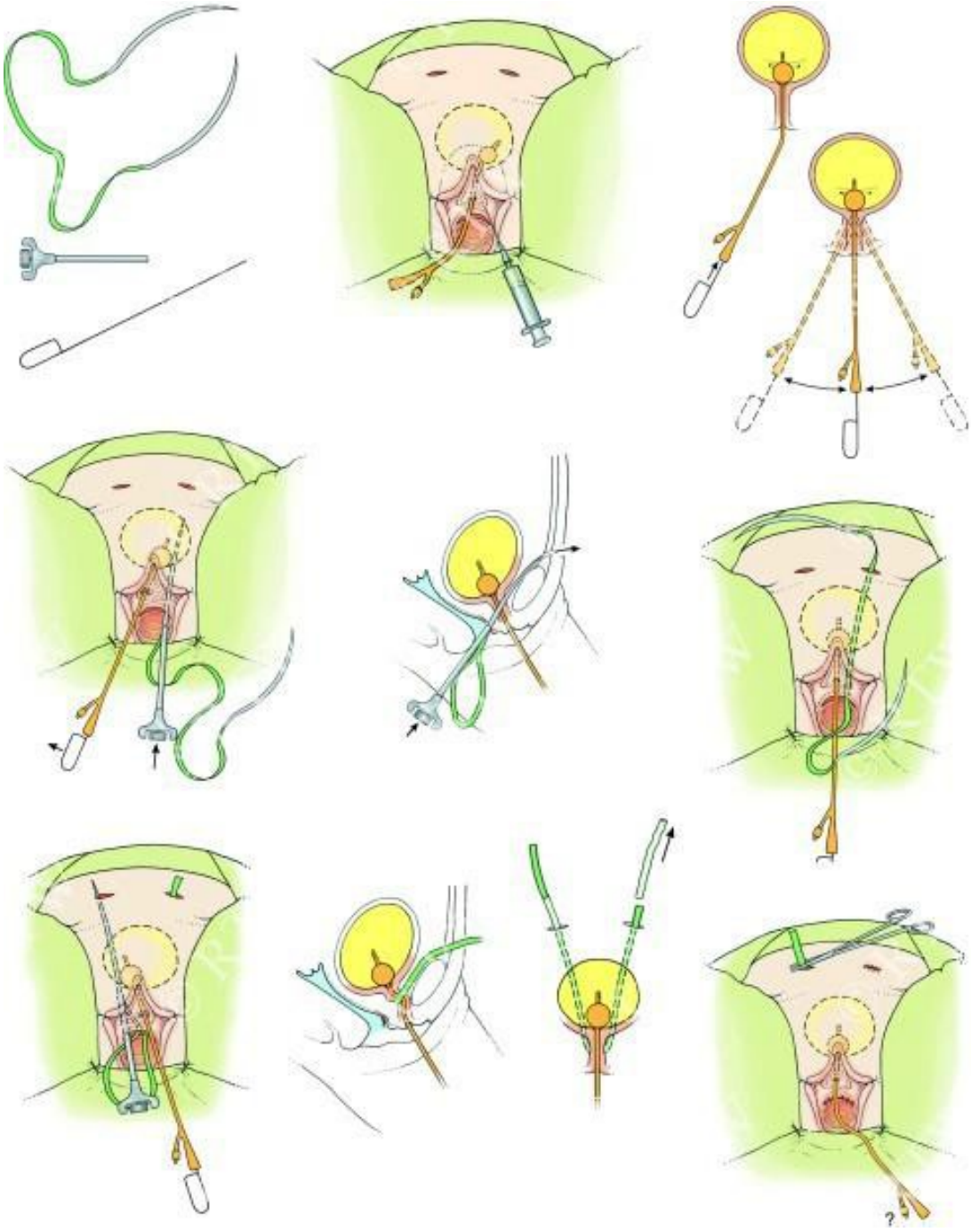


Figure 1.7.6. ***The TVT – tension-free vaginal tape procedure***
Reproduced with permission from Turner-Warwick T and Chapple C. Functional reconstruction of the female genito-urinary tract. Blackwell 2002.

1.7.5.3 Comparative data – Bulking agents versus surgery

Table 1.7.3 demonstrates the outcomes of bulking agents *versus* surgery. Corcos [205] compared Contigen™ injections with surgery (needle suspension (n=6), Burch colposuspension (n=19) and PVS (n=29)) and demonstrated significantly improved success rates as defined by a <2.5g 24 hour pad weight test for surgery over bulking agents (72.2% *versus* 53.1% respectively) after 12 months follow-up. Despite this, there was no significant difference in patient satisfaction scores or quality of life. Similarly, the absence of urodynamic stress incontinence has been demonstrated in 81% of patients following a pubovaginal sling over 9% of patients at 6 months follow-up following Macroplastique®; Macroplastique® was also more expensive but resulted in fewer complications [206]. More recently, Gaddi [207], in a retrospective cohort study of patients with recurrent SUI following mid-urethral sling surgery, demonstrated that 89% undergoing repeat sling surgery demonstrated an absence of urodynamic stress incontinence as compared with 62% of those who received urethral bulking agent injection (Coaptite®, Contigen™ or Macroplastique®), without any significant differences in complications between the two groups.

1.7.5.4 Comparative data – sling/tape procedures versus NTR

Table 1.7.4 demonstrates the outcomes for slings versus NTR. The SiSTER trial [208], a multicentre randomized study compared rectus fascial PVS to BC and found objective success was higher for the sling over colposuspension (66% versus 49% respectively ($p<0.001$)). Despite this, the sling was also associated with a higher likelihood of morbidity including urinary tract infections, voiding dysfunction (14%) and *de novo* urinary urgency (3%). Demerici [209] compared autologous rectus fascial PVS to the BC, demonstrating a 94% versus 88% objective cure rate, which did not reach statistical significance at 12 months follow up. Meanwhile, a further study showed a 92.8% versus 87.8% objective cure rate for the PVS as compared to the BC ($p<0.05$), while retention was demonstrated in 7.1% of the PVS cohort [210].

There is evidence from numerous randomized controlled trials and systematic reviews that compare the efficacy of MUT to older procedures. Novara *et al* [157], in a

systematic review of 39 randomized controlled trials, concluded that patients treated with retropubic MUT experienced slightly higher continence rates than those patients who had BC, despite a higher risk of bladder injury. In a meta analysis of 3 RCTs, Ogah [211] demonstrated a 79% versus 77% objective cure rate for MUT versus BC.

1.7.5.5 Comparative data – biomaterials used during sling/tape procedures

Table 1.7.5 demonstrates the outcomes for a variety of sling procedures. The rectus fascial PVS has been effective on long-term follow-up. Bai [210] demonstrated that objective cure rates for the autologous fascia PVS and MUT were 92.8% and 90.3% respectively at 12 months follow up. Despite this, other RCTs have demonstrated no significant difference between the two after 24 months of follow-up [212-214]. Basok [215] compared cadaveric fascia lata PVS versus MUT and found a 79% to 70.8% objective cure rate in favour of the PVS, which was not significant.

Paparella *et al* [216] compared transobturator porcine cross-linked collagen dermis slings with MUT. The group demonstrated objective cure rates of 88.2% versus 88.8% for the sling versus MUT respectively; a finding, which did not reach statistical significance. This finding was supported by data from Ugurlucan [217], however the patient cohort in this study was highly heterogenous.

Novara [157] found that retropubic MUT and PVS were similarly effective, while TVT had slightly higher objective cure rates compared to TOT. TOT had a lower risk of bladder and vaginal perforations and storage lower urinary tract symptoms (LUTS).

Table 1.7.3. *Bulking agents versus surgery*

	Contigen™	**Macroplastique®**
Success rate	53.1%	9%
Follow up (months)	12	6*
Success versus NTR and sling	Inferior	Inferior
Complications	*De novo* urgency 12.6%	*De novo* urgency 0% Voiding dysfunction 5%
Studies	1 RCT [205]	1 RCT [206]

Table 1.7.4. *Slings/tapes versus NTR*

	Autologous fascia PVS	**MUT**
Success rate	66-94%	79%
Follow up (months)	12-24	12-48
Success versus NTR	Equivalent	Equivalent
Complications	*De novo* urgency 3-5.9% Voiding dysfunction 7.1-14%	Voiding dysfunction 5.5% *De novo* urgency 8.9% Mesh exposure 3.2%
Studies	3 RCT [208-210]	1 meta analysis [211]

Table 1.7.5*. Biomaterial repair versus PPL MUT*

	Autologous fascia PVS	**Cadaveric fascia**	**Porcine cross-linked collagen (TO)**
Success rate	47.6%-93.7%	79%	88.2%
Follow up (months)	12-24	12	24
Success versus MUT	Equivalent	Equivalent	Equivalent
Complications	Not reported	De novo UUI 22%	Not reported
Studies	4 RCTs [210, 212-214]	1 RCT [215]	1 RCT [216]

PVS. Pubovaginal sling

MUT. Mid-urethral tape

NTR. Natural tissue repair

PPL. Polypropylene

RCT. Randomized controlled trial

TO. Transobturator

UUI. Urgency urinary incontinence

1.7.5.6 Surgery for POP – Apical compartment

Uterovaginal and vault prolapse (after a hysterectomy) results from laxity of the uterosacral and endopelvic fascia [218]. The levator ani group of muscles may also contribute towards the development of prolapse through damage resulting from childbirth. Apical prolapse rarely occurs without a concurrent anterior or posterior prolapse and therefore, a combined repair is common. For patients with a uterus, a hysterectomy is usually performed once reproduction has finished [219]. Table 1.7.6 demonstrates the outcomes for apical compartment surgery.

1.7.5.6.1 Native tissue repair

The native tissue repair of an apical prolapse is similar to the suspension procedures that are performed for SUI. Utero-sacral ligament suspension (USLS) is a technique that aims to attach the vaginal wall to the utero-sacral ligaments using non-degradable sutures to restore the normal vaginal axis. This can be achieved using either a trans-vaginal or trans-abdominal approach. Various retrospective cohort studies demonstrate success rates in the range of 48%-96% (mean 85) [220], while the rate or ureteric injury with this procedure is in excess of 10% of cases [221]. A similar procedure, sacro-spinous ligament suspension (SSLS) attaches the vault of the vagina to the sacro-spinous instead of the utero-sacral ligaments using a trans-vaginal approach. Success rates of between 81%-97.6% are quoted in the literature, while recurrent prolapse commonly affects the anterior compartment [220], The limitations of this procedure are that of *de novo* anterior compartment prolapse resulting from increased intra-abdominal pressure [222]. This approach can avoid ureteric injury, however there is a significant risk to the pudendal nerve. Barber *et al* demonstrated no significant difference in the outcomes between USLS and SSLS at 2 years post-operatively in the context of the OPTIMAL RCT [223].

1.7.5.6.2 Biomaterial repair

The treatment of apical prolapse using biomaterials can either be achieved through the trans-vaginal or trans-abdominal route. Trans-vaginal mesh repair of apical prolapse fixes the mesh to the sacro-spinous ligament, while the abdominal route (sacrocolpopexy (SC) fixes the vaginal wall to the longitudinal ligament overlying the sacrum. SC is commonly performed for patients who have previously undergone a

hysterectomy and develop apical prolapse.

The recurrence rates of the trans-vaginal mesh repair of an apical prolapse range between 0-13%, the major complication being exposure of the mesh in up to 15% of cases [220]. One RCT reported significantly lower success rates after 2 years of follow-up for trans-vaginal mesh repair *versus* SC (42% *vs* 77% respectively) [224]. In this study, the rate of mesh exposure was 13% in the trans-vaginal mesh group, compared with 2% in the SC group (p=0.7)

With SC, the mesh is placed without tension and is located outside the peritoneum, which may explain the lower risk of developing mesh exposure. Recurrence of the apical prolapse has been reported to occur in between 0%-22% of reported series [220]. In a Cochrane review, Maher *et al* analysed 3 RCTs that compared SSLS to SC, demonstrating success rates of 84.7% and 96.4% respectively over a mean follow-up period of 2 years [225]. Given the limitations of trans-abdominal surgery as compared to trans-vaginal surgery, it is not surprising that the complications following SC are more severe, including bladder injury (3.1%), bowel injury (1.6%), ureteric injury (1%) and a transfusion rate of 4.4% [226]. Therefore, despite a higher failure rate, the trans-vaginal approach is a preferred option for patients with poor general health.

Culligan *et al* [227] randomized patients to receive either cadaveric fascia lata or polypropylene mesh in SC. No apical recurrence occurred in either group after 12 months of follow-up, however, recurrence in either the anterior or posterior compartment were statistically more likely to occur with cadaveric fascia as compared to mesh (32% *vs* 9% respectively). The authors described the 5-year outcomes of this RCT in 2011 [228] and report similar recurrence rates, while exposure of the mesh occurred in 3.7% of cases and none in the cadaveric fascia group.

1.7.5.7 Surgery for POP – Anterior compartment

The anterior vaginal wall is the most frequently affected vaginal compartment by prolapse. Damage to the levator ani of the pelvic floor leads to downward pressure of the anterior vaginal wall from the intra-abdominal organs [72]. A repair can either be performed by plication of the vaginal wall only, or plication followed by augmentation

using biomaterials. Table 1.7.7 demonstrates the outcomes for anterior compartment surgery.

1.7.5.7.1 Native tissue repair

Prolapse of the anterior compartment is commonly treated using an anterior colporrhaphy. This involves dissection of the epithelium free of the vaginal wall (muscularis mucosa/adventitia), followed by plication of the paravaginal layers of tissue in the midline. Slowly degradable sutures are used for this and finally the vaginal epithelium is closed in a similar fashion.

Anterior colporrhaphy is the traditional approach to repair POP affecting the anterior compartment. Large volume case series demonstrate the long-term success of these procedures in excess of 80% [229]. However, Olsen [40] demonstrated that the re-operation rate in the long-term is in excess of 29% following the index surgery. As a result of this, the use of biomaterials to augment the repair site became increasingly popular amongst clinicians.

The paravaginal repair, aims to reinforce the attachment of the paravaginal tissues to the attachments along the ATFP. While no RCTs exist, which describe the outcomes of paravaginal repair in isolation, several large case series describe success rates of between 67%-100% for a vaginal approach and 75%-97% [229].

1.7.5.7.2 Biomaterial repair

A repair can be reinforced with biomaterials, either as an on-lay over the site of the colphorrhaphy, or as part of a guided mesh kit. A plethora of RCT data and meta analyses exist to support a host of synthetic and biological materials over the traditional methods of colporrhaphy. The 2013 Cochrane review by Maher *et al* [225] provides one of the most robust reviews in this area. 10 RCTs compared polypropylene trans-vaginal mesh repair to NTR in anterior prolapse and demonstrated success rates of 86% and 54% respectively (RR 3.3) after a minimum of 12 months follow-up (maximum 36 months). The mesh groups demonstrated a significantly greater proportion of complications, including chronic pain, bladder injury (2.4% *versus* 0.3% in NTR). Mesh exposure was found in 11.4% of patients and

as a whole, 6.8% of patients required remedial surgery for this.

Owing to the risks associated with non-biodegradable polypropylene mesh, degradable mesh materials have been assessed. The meta-analysis by Maher *et al* [225] described the outcomes of two RCTs [230, 231] that reported the use of degradable polyglactin mesh materials compared with NTR over a 12 to 24 month follow-up period. NTR was associated with a greater proportion of recurrent anterior compartment prolapse than the polyglactin mesh (RR 1.39; 95% CI, 1.02 – 1.90), with only one case of mesh exposure reported between the two studies. The authors also reported that NTR had a higher risk of recurrence than the use of a porcine dermis mesh inlay repair (RR 2.08; 95% CI, 1.08 – 4.01) in the pooled data of 3 RCTs [232-234], and a further 2 RCs [235, 236] that demonstrated success rates ranging from 38% to 93% for porcine dermis repair. However there was no significant difference in the post-operative patient self-reporting of prolapse after either polyglactin mesh (RR 0.96) or porcine dermis (RR 1.21).

Other biological repair materials have been assessed for the augmentation of a repair in anterior compartment prolapse. Feldner [237] compared small intestinal submucosa (SIS) grafts with NTR and demonstrated a 86.2% anatomic cure compared to 59.3% respectively (p=0.003) over a follow-up period of 12 months. No patients demonstrated exposure of the graft and there were similar episodes of dyspareunia between the two groups. Meanwhile, Gandhi [238] and co-workers randomized patients to receive cadaveric fascia lata augmentation versus NTR and demonstrated a 79% *versus* 71% anatomical success rate, which did not reach statistical significance. The use of bovine pericardium as a repair graft was studied in a RCT by Guerette [239]. After 2 years of follow-up, the anatomical success rates were 76.5% for the bovine pericardium graft group and 63% for NTR (p=0.509).

1.7.5.8 Surgery for POP – Posterior compartment

Rectocoeles are the most common posterior vaginal wall defect. Posterior prolapse often occurs in combination with prolapse of other vaginal compartments and this defect is caused by divarication of the levator ani muscles. This can lead to bowel symptoms as the bowel protrudes through the fascial defect of the posterior vagina

towards lower vagina or perineum. Table 1.7.8 demonstrates the outcomes for posterior compartment surgery.

1.7.5.8.1 Native tissue repair

The traditional repair techniques of colporrhaphy or plication in the midline are commonly used in the repair of posterior compartment prolapse. Site-specific posterior repair techniques are used for the approximation of the discrete fascial defects as described above, using interrupted sutures. The review by Karram and Maher [240] demonstrated similar outcomes for each of these procedures, with anatomical success rates of 83% for both colporrhaphy and site-specific posterior repair, with identical rates of dyspareunia (18%).

1.7.5.8.2 Biomaterial repair

The procedure for augmenting the posterior repair site with biomaterials is similar to that used for the treatment of anterior compartment prolapse. However, no trial data exists that independently compares the polypropylene mesh used in anterior compartment or apical prolapse with NTR. A modified version of the SC to repair apical prolapse with co-existing posterior compartment prolapse, using polypropylene mesh has demonstrated anatomic success rates of 90% over 12 months, in the largest series by Su *et al* [241].

The degradable mesh, polyglactin has not demonstrated a significant improvement in success rates as compared to NTR alone in RCT data over 12 month follow-up. Success rates of 89.6% and 91.8% respectively were found by Sand [230], while there were no significant graft-related complications in this study. Furthermore, the meta-analysis by Maher [225] analysed the data of two RCTs that compared porcine small intestinal submucosa with follow-up in excess of 12 months [242, 243]. Anatomical success was 79% and 90% for biomaterial *versus* NTR respectively. Meanwhile, cross-linked porcine dermis augmented repair was compared with NTR in one RCT, demonstrating posterior compartment success rates as assessed with the POP-Q score of 87% and 60% respectively over a 3 year follow-up period; exposure was demonstrated in 4.4% of cases [244].

1.7.5.9 POP surgery - Conclusions

For apical prolapse, SC offers significantly superior success rates as compared with NTR, with polypropylene mesh demonstrating fewer recurrences than cadaveric fascia lata. Furthermore, SC is superior to trans-vaginal mesh repair of apical defects and associated with significantly fewer episodes of mesh exposure. The anterior compartment is the most frequently affected by prolapse. Here, augmentation with polypropylene mesh demonstrates greater success rates than NTR, despite being associated with a high risk of mesh exposure (10%), which is significantly higher than the use of other biomaterials, including degradable mesh and porcine dermis. Both porcine dermis and small intestinal submucosa are associated with higher success rates than NTR alone, with comparable recurrence rates to polypropylene mesh in anterior repair. However, there is no evidence to suggest a benefit for the use of biomaterials in posterior compartment prolapse, where rates of exposure are high.

Table 1.7.6. *Outcomes of biomaterials used in apical compartment surgery versus NTR and SC*

	Trans-vaginal PPL	**SC PPL**	**SC cadaveric fascia**
Success rate	42%	84.7%-96.4%	69%
Follow up (months)	24	24-60	60
Success versus NTR	Equivalent	Superior	N/A
Success versus SC PPL	Inferior	N/A	Inferior
Complications	Exposure 13%	Exposure 5%	None reported
Studies	1 RCT [224]	1 Meta-analysis (4 RCTs) [225]	1 RCT [227] 1 follow-up [228]

Table 1.7.7. *Outcomes of biomaterials used in anterior compartment surgery versus NTR*

	PPL mesh	**Degradable mesh**	**Porcine dermis**	**SIS**	**Cadaveric fascia**	**Bovine pericardium**
Success rate	86%	42%-75%	38%-93%	86.2%	79%	76.5%-85.7%
Follow up (months)	12-36	12-24	12-60	12	13 (median)	12-24
Success versus NTR	Superior	Equivalent - Superior	Equivalent – Superior	Superior	Equivalent	Equivalent
Complications	Bladder injury 2.4% Exposure 11.4% Dyspareunia 7%	Erosion 2.8%	Exposure 4.4%	No exposuse Dyspareunia 17%	None reported	None reported
Studies	1 meta-analysis (10 RCTs) [225]	1 meta-analysis [225] of 2 RCTs [230, 231]	1 meta-analysis [225] and 2 RCTs [235, 236]	1 RCT [237]	1 RCT [238]	1 RCT [239]

Table 1.7.8. *Outcomes of biomaterials used in posterior compartment surgery versus NTR*

	PPL mesh*	**Degradable mesh**	**SIS**	**Porcine dermis**
Success rate		89.6%	54%-88%	87%
Follow up (months)		12	12	36
Success versus NTR		Equivalent	Inferior	Equivalent
Complications		None reported	No complications	Exposure 4.4% Vaginal stenosis 10%
Studies		1 RCT [230]	2 RCTs [242, 243]	1 RCT [244]

* Trans-vaginal PPL mesh not independently assessed for posterior compartment prolapse

PPL. Polypropylene

NTR. Native tissue repair

SC. Sacrocolpopexy

SIS. Small intestinal submucosa

Data pooled from findings of included controlled trial and cohort study data.

1.8 Complications of biomaterials that are used in SUI/POP

Autologous fascia is a common repair material. Prior to the advent of synthetic mesh materials for pelvic floor repair, these tissues were associated with high anatomical and functional success rates. The problem with these tissues are those of donor site morbidity that is associated with a significant incision on the abdominal wall or thigh to harvest the tissue [245]. In an attempt to overcome this problem, other biological materials have been extensively investigated, including allografts and xenografts. Although these tissues are readily available, widespread use is limited due to issues with infection and an unpredictable host response that is often associated with a varied degree of altered mechanical behavior. Synthetic materials gained popularity in the surgical community, particularly for use in hernia repairs and these materials were rapidly adapted for use in pelvic floor reconstruction

In 2008, following a rise in the reports of serious complications received by the US Manufacturer and User Facility Device Experience (MAUDE) database, the US Food and Drug Authority (FDA) issued the first of several notifications on the safety of these devices and 'kits' [246]. In 2011, the FDA issued a second notification [247] to warn patients of the risks associated with trans-vaginal mesh, which stimulated the first of many class action lawsuits in the US and has led to the eventual withdrawal of several mesh materials from the market.

The exact reason behind why these complications occur is not completely understood and probably reflects our current appreciation of the mechanisms behind the development of POP/SUI itself and the determinants of a successful outcome.

In this section, the host response and physical properties of biomaterials following their implantation into the body are discussed.

1.8.1 Defining biomaterial complications

The joint International Urogynaecology Association (IUGA) and the International Continence Society (ICS) working group on complications terminology [248] proposed a new classification of complications in an attempt to standardize the reporting of such events. The term device 'erosion' ("state of being worn away, as by friction or pressure"), was replaced by terms with a more appropriate anatomical description, such as 'exposure' (a condition of displaying, revealing, exhibiting, or making accessible) and 'extrusion' (passage gradually out of a body structure or tissue). These complications can be associated with other post-operative problems following surgery that uses biomaterials, e.g infection, chronic pain, voiding dysfunction and dyspareunia. MUT placement can also exacerbate or develop *de novo* urinary urgency or urgency urinary incontinence in 27.7% and 13.7% of patients respectively at long-term follow-up [249]. Dyspareunia and chronic pain following SUI surgery can be the most distressing complication for patients [250] and is potentially irreversible. For trans-vaginal mesh in the treatment of POP, dyspareunia can occur in up to 20% and 64% of patients undergoing an anterior and posterior repair respectively [251].

Mesh exposure/extrusion is estimated to occur in 10% of patients undergoing POP repair with mesh [252] and to a lesser extent following a trans-vaginal tape (TVT) insertion for SUI [253]. These findings may in fact be underestimates of the problem, given that the duration of clinical follow-up in many reported series is short and that mesh exposure can take years to occur [254].

Most cases of vaginal mesh exposure are felt, before they are seen and often present with pain and dyspareunia. It is important therefore to exclude mesh exposure in these patients using a combination of a detailed clinical history and examination with other radiological (ultrasound is more efficacious than MRI) or direct imaging methodologies. Clearly, patients with mesh exposure may be asymptomatic and do not warrant surgical management, just as small exposures (<1cm) without any complicating factors can be treated conservatively with topical oestradiol alone. Surgical treatment of the exposed or extruded mesh is technically difficult and patients should be appropriately counseled about the recurrence of SUI, fistulae and chronic pain.

MUT failure has been defined in 4 ways immediately after surgery and can also be applied to sling/tape surgery in general [255]:

1. Failure to cure SUI.
2. SUI cured, but *de novo* overactive bladder (OAB) symptoms.
3. SUI not cured, and *de novo* OAB symptoms.
4. New symptoms or complications, e.g dyspareunia, exposure, chronic pain.

When there is recurrence of the original symptoms following a 12 month period of symptomatic improvement following MUT insertion, then these patients are regarded as having symptom recurrence rather than failure [255].

The recent report by IUGA and the ICS [248] brought standardization to terminology and classification related to mesh surgery, which includes an assessment of the category, timing and site of mesh complications (table 1.8.1). Standardized definitions, such as the IUGA/ICS report allow inter-study outcome comparisons to be made, which at present is difficult. Despite this, the IUGA/ICS terminology is complex and does not form part of routine assessment.

Table 1.8.1. *IUGA/ICS classification of complications related directly to insertion of prosthesis or grafts in female pelvic floor surgery*

	Category			
General Description	**A (Asymptomatic)**	**B (Symptomatic)**	**C (Infection)**	**D (Abscess)**
1. Vaginal: No epithelial separation. Includes Prominence or contraction	**1A:** Abnormal prosthesis or graft finding on clinical exam	**1B:** Symptomatic e.g. unusual discomfort/pain; dyspareunia; bleeding	**1C:** Infection (suspected or actual)	**1D:** Abscess
2. Vaginal: Smaller exposure (<1cm)	**2A:** Asymptomatic	**2B:** Symptomatic	**2C:** Infection	**2D:** Abscess
3. Vaginal: Larger exposure (>1cm), or any extrusion	**3A:** Asymptomatic. 1-3Aa if no prosthesis or graft related pain	**3B:** Symptomatic. 1-3B(b-e) if prosthesis or graft related pain	**3C:** Infection. 1-3B(b-e) if prosthesis or graft related pain	**3D:** Abscess. 1-3D(b-e) if prosthesis or graft related pain
4. Urinary tract: Compromise or perforation including prosthesis perforation, fistula and calculus	**4A:** Small intraoperative defect. E.g bladder perforation	**4B:** Other lower urinary tract complication or urinary retention	**4C:** Ureteric or upper urinary tract complication	
5. Rectal or bowel: Compromise or perforation including prosthesis perforation and fistula	**5A:** Small intraoperative defect (rectal or bowel)	**5B:** Rectal injury or compromise	**5C:** Small or large bowel injury or compromise	**5D:** Abscess
6. Skin and/or musculoskeletal: Complications including discharge, pain, lump or sinus	**6A:** Asymptomatic, abnormal finding on exam	**6B:** Symptomatic e.g discharge, pain or lump	**6C:** Infection e.g sinus	**6D:** Abscess
7. Patient: Compromise including haematoma or systemic compromise	**7A:** Bleeding complication including haematoma	**7B:** Major degrees of resuscitation or intensive care	**7C:** Mortality (additional complication-no site available – S0)	

Time (Clinically diagnosed)			
T1: **Intraoperative – 48 hours**	**T2:** 48 hours – 2 months	**T3:** 2 – 12 months	**T4:** >12 months

Site				
S1: Vaginal: Area of suture line	**S2: Vaginal:** Away from area of suture line	**S3: Trocar passage** Exception: intra-abdominal (S5)	**S4: Other** skin or musculoskeletal site	**S5: Intra-abdominal**

Pain	
Grade of pain	**Symptoms**
a	Asymptomatic or no pain
b	Provoked pain only (during vaginal examination)
c	Pain during intercourse
d	Pain during physical activities
e	Spontaneous pain

1.8.2 Mesh complications

1.8.2.1 Host response to implantation

Following the implantation of any non-absorbable material, the host response takes the following time course [256] :

1.	Initial blood-material interaction	Minutes to hours
2.	Provisional matrix formation	Hours to days
3.	Acute inflammation	Days to one week
4.	Chronic inflammation	< Two weeks
5.	Granulation tissue development	Weeks to months
6.	Foreign body reaction (FBR).	Weeks to months
7.	Fibrosis	Months to years

Immediately following implantation, a biofilm rich in mitogens; cytokines and other growth factors surrounds the mesh. These factors activate cell populations involved in the inflammatory and wound healing responses. Polymorphonuclear leucocytes indicate an acute inflammatory response, which usually subsides within a week, followed by a chronic inflammatory response, which lasts for no more than 2 weeks if a material is biocompatible. Granulation tissue forms around the implanted mesh and neovascularization begins to occur followed by macrophage infiltration and foreign body giant cells, which constitutes the FBR and indicates the end of the inflammatory phase of healing. The granulation tissue is replaced by collagen, deposited by fibroblasts and fibrosis ensues [256].

An initial inflammatory response allows host cell infiltration, however a prolonged chronic course is clearly undesirable. Polypropylene mesh produces a vigorous FBR [257], which never completely abates. The FBR is associated with an up-regulation of matrix metalloproteinases and pro-inflammatory cytokines, which can lead to persistent remodeling and inflammation at the mesh-host interface, which could be one explanation for exposure [258]. Furthermore, women who developed mesh exposure were found to have cytokine levels 3-times greater pre-operatively than

women without mesh exposure [259], which can explain why some women develop the complication whereas others do not while the contraction of collagen over time can lead to prominence or contraction of the tape [260].

1.8.2.1.1 Mesh structure

Polypropylene is considered a relatively biocompatible thermoplastic polymer, however complications do occur in practice. Greater porosity is purported to be associated with a greater degree of cell penetration, neovascularization and matrix production [261]. A smaller pore size on the other hand, is associated with chronic inflammation due to encapsulation, which can lead to chronic infection and exposure [262]. Filament type (monofilament versus multifilament) has been demonstrated to have an impact on rates of complications, particularly the observed exposure rates when multifilament meshes are used. The concern with multifilamentous meshes is that micro-organisms can colonise the areas between fibres.

1.8.2.1.2 Biomechanics

The mechanical mismatch between the rigid PPL mesh and the elastic paravaginal tissues, which are under constant dynamic distension can lead to plastic deformation of the mesh [263], which can then gradually pass out of a body structure. This is highlighted by recent *in vivo* work that demonstrates exposure of PPL mesh over 3 months when implanted trans-vaginally in sheep models, whereas no such response occurs when this material is implanted abdominally [264], illustrating the site-specific response to this material.

Recently, novel devices for the more accurate dynamic measurement of intra-vaginal pressures have been developed [265] and highlight the importance of the mechanical properties of the mesh, which may previously have been under-appreciated.

1.8.2.1.3 Material degradation

Synthetic materials are selected for their predictable properties. These properties are well studied *in vitro*, however *in vivo* long-term studies are lacking in the literature. In a study of 100 patients with mesh related complications (infection and exposures), the explanted failed meshes were examined. Cracking and material degradation was

demonstrated in 49.33% of these [266].

1.8.2.2 Clinical complications - mesh toxicity

As a structure, polypropylene has the potential to degrade to form toxic aldehydes and carboxylic acids, demonstrated in a rat model following prolonged exposure and extreme temperatures [267]. Such conditions are unlikely to be encountered in human subjects, however these findings highlight the importance of avoiding autoclave or gamma radiation during the sterilization process. Carcinogenicity has also been investigated in animal models, demonstrating potential for polypropylene materials to be associated with sarcoma [268, 269], however, no such carcinogenicity has been ever demonstrated in human subjects [270].

1.8.2.3 Clinical complications - infection and chronic pain

Infection following mesh implantation may be associated with mesh exposure. As previously discussed, infection is more likely to occur with microporous or multifilamentous mesh materials and can result from a variety of Gram positive and negative, aerobic and anaerobic bacteria with an incidence of 0-8% in trial data [271]. Methods to reduce levels of mesh contamination include peri-operative antibiotics, skin antisepsis and limitation of tissue dissection [272]. Mesh infection generally requires excision of the entire mesh material.

Chronic pain in POP surgery is estimated to occur in 1.9-24.4% of patients [273] , whereas 40% of patients experience some degree of thigh or groin pain with mid-urethral slings [274]. If conservative therapies with analgesics fail, treatment is with mesh excision following appropriate counseling regarding the inherent risks with such a procedure.

Dyspareunia has been demonstrated to occur in 9.1% of patients undergoing mesh surgery for anterior prolapse [275], with lower rates of dyspareunia resulting from mid-urethral tapes. One mechanism behind the development of *de novo* dyspareunia is 'para-urethral banding', that can result from the bands of para-urethral folds of the anterior vagina becoming palpable. The exact mechanism of this is as yet unreported [250]. Mesh incision or excision can alleviate symptoms of dyspareunia [276].

1.8.2.4 Clinical complications - voiding dysfunction

As a result of surgery using mesh implants, patients can develop either incontinence or post-operative urinary retention. *De novo* SUI can result following tran-svaginal mesh repair of prolapse and this has been demonstrated to be successfully treated either inter-operatively or post-operatively with mid-urethral slings [277]. Following mid-urethral tape procedures for SUI, it is well recognized that acute urinary retention can occur in roughly 3.1-32% of patients in the acute phase [278] and therefore patients are usually counseled that this could occur and are commonly taught intermittent self-catheterization pre-procedure. Urinary retention is thought to develop due to the tape being excessively tight and can be treated either conservatively with self-catheterization or with operative intervention. This can involve either sling incision [279, 280] or formal urethrolysis [281]. Furthermore, delayed *de novo* overactive bladder type symptoms can occur following treatment for SUI, which may be related to a degree of relative bladder outlet obstruction as a result of mesh tension [282]. Such patients require exclusion of urinary tract infection and mesh exposure as a cause of their symptoms, followed by urodynamic assessment to identify whether detrusor overactivity or bladder outflow obstruction is the cause. For those patients with *de novo* detrusor overactivity, treatment options include anticholinergic therapies, beta-3 adrenoreceptor agonsists (mirabegron), or intravesical botox with or without the need to perform clean intermittent self-catheterization (CISC).

1.8.3 Biomaterial complications

The complications that are observed with the use of biological grafts for use in pelvic floor reconstruction can occur as a consequence of several factors. The tissues are processed and sterilized for use in order to prevent any transmission of contaminating bacteria or fungi. The sterilization can affect the structural components of the graft. Furthermore, the response of the host to biological materials may involve chronic inflammation and macrophage infiltration and this can lead to complications. In this section, we discuss the effect of these steps on the integrity and properties of the graft and try to correlate these with the surgical outcomes.

1.8.3.1 Tissue processing

Allografts and xenografts undergo processing to remove the cellular components that are associated with the host response. Unfortunately, this can denature the complex peptides and other molecules, such as cytokines and growth factors that play an important role in the integration of the material. Newer biological grafts, such as cross linked porcine dermis and SIS, undergo much less robust processing techniques and therefore retain many of the molecules, such as glycosaminoglycans and fibrillar proteins than do cadaveric fascia. As a result, tissues such as cadaveric fascia undergo a degree of degradation, prior to implantation; following which a variable degree of chronic inflammation occurs with the subsequent formation of scar tissue. Meanwhile, tissues with a relatively retained ECM component are able to release certain bioactive molecules, such as platelet derived growth factor, growth factors and other cytokines to influence the initial healing phase of a wound bed following implantation [283, 284]

1.8.3.2 Integration and degradation

While mesh material that is explanted from animal models demonstrates incorporation into scar tissue, cadaveric fascia becomes extensively thinned or is even absent altogether, with little evident cellular infiltrate [285].

The technique of collagen cross-linking, using chemicals was introduced in order to overcome this problem. It is observed that these tissues retain their strength and resist degradation to a much greater degree. The disadvantage to this method is that of poorer integration into the host and a FBR occurs [286], with resultant encapsulation, chronic inflammation and inherent degradation of the tissues [287]. This factor may explain why cross-linked tissues result in higher rates of exposure than cadaveric tissues.

Contemporary biological grafts undergo constructive remodeling [288]; a process that results in organized tissue formation following controlled degradation and cellular infiltration. Autologous fascial grafts also undergo constructive remodeling, as the cellular component of these tissues has not been removed. While an initial mild inflammatory response occurs, an FBR does not occur, thus cellular ingrowth and

neovascularization can occur [200] in addition to synthesizing an organized matrix [289].

The macrophage response is an important mechanism involved in the remodeling processes, which a biomaterial undergoes following implantation. Two distinct macrophage phenotypes exist: type 1 (M1) and type 2 (M2). M1 macrophages are associated with a cytotoxic and inflammatory type response, the aim of which is to destroy certain pathogens or tissues recognized as foreign [290]. M2 macrophages, however lead to the process of constructive remodeling as described earlier. Therefore, a material that results in an M1 macrophage response is associated with chronic inflammation and degradation, while one that leads to an M2 response results in an organized cellular structure. Tissues that fail to initiate either macrophage response can lead to encapsulation.

The exact mechanism that leads to a specific macrophage response is likely to be due to the release of proliferative factors that occur following implantation into the wound bed. Therefore, tissues such as autologous fascia and grafts that have undergone minimal processing techniques will lead to an M2 response. Chemical cross-linking prevents the degradation and release of these mediators and therefore an M1 response is initiated [291]. A summary of the key features of each material is presented in table 1.8.2.

1.8.4 The ideal material

In a search for the ideal material, several key components have been proposed. Although no current material possesses all of these properties, several repair materials are available that focus on certain specific areas of the host response. Karlovsky et al [292] suggested that an ideal material should be sterile, inert, not overly inflammatory, non-immunogenic, durable and easily accessible. Although those materials that result in an acute inflammatory response that persists to a chronic phase (M1 macrophage response) may be associated with infection and exposure [293], one that does not initiate an inflammatory response at all may lead to encapsulation [294].

Materials that are biodegradable are associated with lower levels of infection and chronic pain however, these must undergo controlled degradation over a period that allows sufficient tissue remodeling (M2 macrophage response) with production of ECM, angiogenesis and fibroblast ingrowth [291]. Candidate materials for implantation should also handle well and have an ability to retain sutures in order to be adequately implanted surgically. Human fibrin glue devices, such as Tiseel™ (Baxter International) are composed of clotting agents, including factor XIII, fibronectin and thrombin and are used promote haemostasis, however, activation of the clotting cascade also results in tissue remodeling and fibrosis. The degree to which fibrosis and an inflammatory response occurs has not been established, however its application between a repair material and healthy tissues could theoretically avoid excessive inflammation at the mesh-tissue interface.

Table 1.8.2. *Comparison of biomaterials used in pelvic floor reconstruction*

Class	Types	Degradation	Advantages	Disadvantages
Autograft	Fascia	Months-years	Minimal inflammatory response	Donor site morbidity.
			Constructive remodeling	Insufficient tissue
Synthetic	Polypropylene	Many years	Durable	High number of complications
			Greatest evidence base	Poor evidence base
	Polyglactin	Months	Durable	
			Degradable	
Allograft	Cadaveric fascia	Months	Available	Chronic inflammatory response
	Cadaveric dermis	Months	No donor site morbidity	Infection transmission
Xenograft	Porcine dermis	Years	Available	Poor integration
			Durable	FBR
	SIS	Months	Constructive remodeling	Poor mechanical strength

SIS. Small intestinal submucosa

FBR. Foreign body reaction

1.9 Regenerative medicine and tissue engineering techniques for pelvic floor reconstruction

The concept of tissue engineering is used interchangeably with the term 'regenerative medicine'. However, many experts would argue that these two fields are entirely different entities. Regenerative medicine is defined by Daar [295] as:

> *"An interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma and ageing. It uses a combination of several converging technological approaches, both existing and newly emerging, that moves it beyond traditional transplantation and replacement therapies. The approaches often stimulate and support the body's own self-healing capacity. These approaches may include, but are not limited to, the use of soluble molecules, gene therapy, stem and progenitor cell therapy, tissue engineering and the reprogramming of cell and tissue types."*

Tissue engineering is defined by Langer [296] as:

> *"An interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve [Biological tissue] function or a whole organ".*

Clearly, there are many similarities between these two fields, however it is generally accepted that tissue engineering places an emphasis on the use of scaffold materials combined with cells, while those involved in regenerative medicine focus on stem cells.

1.9.1 Regenerative medicine approaches

The functional urethral sphincter relies upon the presence of both striated and smooth muscle components, in addition to a functional nerve supply to effectively resist incontinence. Owing to the poorly sustained outcomes of urethral bulking agent injections, there is interest in the use of stem cells to functionally regenerate the urethral sphincter in those with ISD. Cells from different sources have been injected into the urethral sphincter of small animals in pre-clinical studies and demonstrate promising results. The mesenchymal stem cells (MSCs), which are used

as the cell source in a vast majority of these studies are commonly derived from bone, muscle or adipose tissue. MSCs share several characteristics such as long-term replicative potential and self-renewal, maintenance of undifferentiated properties and multi-lineage differentiation ability [297].

1.9.1.1 Bone marrow derived stem cells

Bone marrow-derived stem cells (BMDSCs) were the first MSCs to be studied in a urology context by Drost *et al* [298] studied the implantation of BMDSCs into the bladder musculature with 5-azacitidine as a myogenic differentiation stimulator. Stem cells differentiated into muscle cells verified by the expression of striated and smooth muscles antigens. Kinebuchy *et al* [299] injected BMDSC, labelled with green fluorescent protein into the urethral sphincter of Sprague-Dawley rats, which were rendered incontinent by urethrolysis. After 13 weeks, fluorescent imaging demonstrated that the skeletal muscle component of the urethral sphincter was regenerated, however the VLPP of the urethra was not improved. Corocos *et al* [300] found similar regenerative outcomes using BMDSCs injected into the urethral sphincter of rats after pudendal nerve transaction and showed significantly improved VLPP 4 weeks after injection. While BMDSC demonstrate promising regenerative properties in pre-clinical studies, the biopsies for bone marrow that would be required in women are painful, frequently requiring general or spinal anaesthesia and often only a low number of BMDSCs are isolated [301].

1.9.1.2 Muscle derived stem cells

Muscle-derived stem cells (MDSCs) can be obtained under local anaesthesia. They can be isolated from different sources; those from striated muscle have shown the greatest regenerative potential in novel disease models. [302]. In addition, these cells can be differentiated into all mesenchymal cell lines, including neuronal and endothelial lineages [303, 304]. This cell type is regarded as the most appropriate source for ISD injection therapies and several studies have found good tissue regeneration, improved VLPP and recovered contractile urethral sphincter function when injecting these cells into the urethral sphincter of different SUI animal models [305-310]. In humans, Mitterberger *et al* [311] reported the outcomes of the dual injection of MDSCs into the rhabdosphincter and fibroblasts into the urethral

submucosa of 123 women with SUI. 79% of women were completely continent with significant improvements in urodynamic parameters, including MUCP, flow rates and post-voiding residual volumes at the end of the 12 month follow-up. Carr *et al* [312] reported that 5 out of 8 women who underwent a peri-urethral injection of MDSCs were subjectively dry and had a negative pad test during follow-up, with a functional onset following injection of between 3 and 8 months and continence was sustained in these 5 patients at the 12 month study endpoint. To obtain a reasonable content of MDSCs, a large biopsy of skeletal muscle (5-30g) is needed, which can be associated with donor site pain and bleeding. Gras *et al* [313] demonstrated that of patients undergoing open vastus lateralis muscle biopsy, 10/40 described pain lasting 2-3 days, 1/40 had a superficial infection and 2/40 developed a haematoma. Despite this, the biopsy required to obtain muscle cells is rapid and less invasive than that used to obtain BMDSC. However, MDSCs differentiate quickly without any stimulation and can differentiate into multinucleated fibres, which do not have the regenerative properties of MSCs, nor do MDSC release paracrine factors (both for nerve and blood vessels regeneration). Furthermore, the slow proliferative potential of MDSC increases the cost of the procedure [314].

1.9.1.3 Adipose derived stem cells

Adipose-derived stem cells (ADSCs) have the particular potential for mesodermal tissue differentiation [315, 316], such as in regeneration and revascularization [317, 318]. Only 40-60% of the adipose tissue is composed of mature adipocytes and ADSCs can easily and quickly be isolated from the stromal vascular fraction of adipose tissue, which is also composed of fibroblasts, macrophages, endothelial cells and hematopoietic cells [314]. These cells proliferate very quickly and do not differentiate without stimulating differentiation medium. ADSCs can differentiate into striated muscle, showing specific markers (desmin, myod1, myogenin, myosin heavy chain) [319] and have been used to regenerate damage skeletal muscle in rabbits, restoring volume and muscular contraction [320]. ADSCs have also been used for urethral sphincter reconstruction to improve continence outcomes in SUI animal models, dueto their capacity to differentiate into smooth muscle cells [316] and to contract and relax under pharmacological stimulation [319]. In humans, Gotoh *et al* [321] reported the outcomes of the trans-urethral injection of ADSC into the

rhabdosphincter in 11 male patients with SUI following a radical prostatectomy. Follow-up was limited to 12 months and significant improvements were noted for pad weight testing, MUCP and for incontinence frequency episodes. Furthermore, 250ml of adipose tissue was aspirated from the abdominal wall with minimal donor site morbidity and cells were rapidly isolated prior to injection.

The outcomes for 5 female patients with SUI were described by Kuismanen *et al* [322] following the trans-urethral injection of ADSCs. Cells were harvested from the abdominal wall, isolated and expanded for 3-4 weeks and were suspended in a Contigen™ matrix prior to injection. At 1 year, 3 patients demonstrated a negative cough test with the bladder filled to 500ml, while the further 2 patients were improved. The 24 hour pad test was significantly reduced in 2/5 patients; those with the greatest degree of urinary leakage pre-operatively. Furthermore, there were improvements in patient reported outcome measures and no significant morbidity resulted following the cell harvest.

The approaches for the treatment of POP differ somewhat to the regenerative medicine techniques used in SUI. With POP, a scaffold is required not only for mechanical support but also to facilitate the integration and proliferation of cells that would usually perish without an adequate substrate for attachment. Unfortunately, PPL meshes seeded with stem cells or fibroblasts have demonstrated poor attachment and proliferation, which may be due to a persistent inflammatory response in the host tissues.

At present, the most commonly used materials for POP repair are non-biodegradable PPL meshes, however the rates of extrusion through patient's tissues with this material are high. Acellular materials such as cadaveric allografts and xenografts have been used to reduce this particular complication, however the long-term failure rates compared to PPL are significantly greater and their degradation is unpredictable [200, 323].

With high failure rates, donor site morbidity and unpredictable degradation using acellular matrices, tissue engineered synthetic bio-degradable polymers have been

investigated as potential repair materials for the treatment of SUI and POP. The rationale behind seeding cells on these scaffolds is to regenerate tissues using cells from an uninjured area of the body. Autologous cells are preferred in this context due to issues with immunogenicity and cross-infection. Classically, these cells are harvested from a patient, isolated and expanded *in vitro* prior to seeding scaffolds with these cells for implantation. Cells that are utilized for regeneration depends on their availability, with stem cells and fibroblasts responsible for many of the biomaterials investigated in the current literature.

1.9.2 Tissue engineering approaches

Scaffolds seeded with cells can be implanted into the patient. Commonly, autologous cells are harvested from the patient to overcome cross-infection and host rejection. Cells are isolated and expanded in culture prior to seeding on the scaffold and subsequent implantation. Thus, the intention is for appropriate integration of the new cells with the host cells. When the intention of tissue engineering is to restore load bearing function, the tensile properties of the implanted biomaterial must be such that the mechanical properties of the weakened tissue are restored, while new supportive tissue is formed to correspond with degradation of the implanted scaffold. Given the complications that occur with the use of PPL mesh, many cell sources and scaffold materials have been investigated. The benefits and complications of each are discussed herein. The majority of studies in this context have not progressed significantly beyond initial pre-clinical stages. A summary of the current tissue engineering approaches to the problem is presented in table 1.9.

1.9.2.1 Cells

Stem cells or fibroblasts do seem a rational source of cells for reconstructive or tissue engineered repair, due to their relative ease of harvest. ADSC, MDSC or fibroblasts can be obtained through local anaesthetic biopsy procedures with minimal reported donor site morbidity or following procedures to remove such tissue that would otherwise be discarded e.g abdominoplasties in which abdominal skin and fat is removed. Autologous cells offer the advantages of overcoming the host tissue response or concerns with disease transmission. BMDSC however, often require painful procedures to obtain sufficient cells for expansion and culture therefore their

use is generally limited.

Of cells cultured *in vitro* on synthetic degradable scaffolds, Roman et al [324] demonstrated that both ADSC and oral fibroblasts (OF) perform similarly in either restrained or unrestrained conditions in terms of ECM component production and cell proliferation. Furthermore, the mechanical properties of these scaffold materials were improved compared to cell free synthetic materials. Moreover, MSCs are better defined, more proliferative and can resist and inhibit myofibroblast differentiation. They can be anti-inflammatory and anti-fibrotic, reducing inflammation and scarring. Also 3D culture increases the anti-inflammatory properties of MSCs and this may be beneficial to the scaffolds [325].

1.9.2.2 Scaffolds

In vivo data has demonstrated the potential of tissue engineered biodegradable scaffolds in rat models rendered incontinent through bilateral sciatic nerve transection. Using woven silk scaffolds seeded with BMDSC, total local collagen production was improved and leak point pressures were similar to continent control subjects [326]. Similar findings have been reported by Cannon et al using MDSC seeded on small intestinal submucosa (SIS) scaffolds that were cultured for 2 weeks prior to implantation [327].

Using poly-L-lactic acid (PLA) scaffolds seeded with OF, Mangera et al [328] demonstrated improved ECM production and comparable biomechanical properties of cells cultured on these materials *in vitro* compared to native tissue. Furthermore, synthetic scaffolds offer the advantage of a predictable degradation and tuning of mechanical characteristics.

Of synthetic matrices of methoxy polyethylene glycol-polylactide-co-glycolide (MPEG-PLGA), seeded with fresh muscle fiber fragments, Boennelycke et al [329] demonstrated new striated muscle formation in explanted samples from rats after eight weeks. Furthermore, Polylactide-co-glycolide (PLGA) scaffolds seeded with human vaginal fibroblasts were implanted in mice and demonstrated a highly proliferative neo-fascia formation in samples up to 12 weeks *in vivo* [330]. Autologous fibroblasts have also been combined with autologous de-epidermised dermis for

implantation into human male urethra for substitution urethroplasty, with three out of five patients demonstrating a patent urethra following the procedure [331].

Polyurethanes have been investigated as potential graft materials, particularly in vascular and bone tissue engineering. The reason for this is due to the elasticity of these polyurethanes and their ability to support cells. Bergmeister et al demonstrated sufficient cell proliferation that continued post-implant. Furthermore, these cylinder shaped scaffolds cultured with endothelial cells for 1 year demonstrated 100% patency at this point [332]. The Badylak group have investigated the use of polyurethanes for repair of abdominal herniae and have shown that polyurethanes offer much improved mechanical profiles than polypropylene and other potential repair materials, but that they can also be fabricated to be more anti-inflammatory [333].

Materials that are biodegradable represent a greater promise than those repair materials that are non-biodegradable. The reasons for this are due to a persistence of an inflammatory response in addition to these materials becoming a focus of infection. Materials that include natural extracellular matrix possess a natural ability to induce tissue remodeling. Small intestine submucosa is such a material, which retains factors including FGF-2 and fibronectin for tissue induction even after sterilization [334-336]. Whilst xenografts do correspond with potential cross-infection, synthetic biodegradable scaffolds seeded with autologous cells could offer a solution to this problem.

Table 1.9. *Summary of pre-clinical studies that investigate tissue engineering for pelvic floor repair*

Author	Cell type	Repair material	Study summary	Outcome
Lu *et al* [337]	MDSC	SIS	*In vitro*. Cells seeded up to 8 weeks.	Cells produced myotubes with contractile activity.
Ho *et al* [338]	MDSC	SIS	*In vitro* and *in vivo*. Cell seeded SIS used to repair rat vaginal wall defects.	Smooth muscle cell differentiation. No fibrosis seen.
Cannon *et al* [327]	MDSC	SIS	*In vivo*. Cell seeded SIS implanted in rat SUI model.	Leak point pressures equivalent to sham controls.
Mangera *et al* [328]	OF	7 scaffolds	*In vitro*. 7 seeded scaffolds. Cell attachment, ECM production and mechanical properties assessed after 2 weeks.	SIS and PLA demonstrated greatest cell attachment, ECM properties and had mechanical properties closest to native tissue.
Zou *et al* [326]	BMDSC	Silk	*In vivo*. Cell seeded scaffolds implanted in rat SUI model.	Leak point pressures equivalent to sham controls.
Boennelycke *et al* [329]	MDSC or fresh muscle fragments	MPEG-PLGA	*In vivo*. Cell/muscle fragment seeded scaffolds implanted subcutaneously in rats	By 8 weeks, MDSC and scaffolds could not be identified, while muscle fragments generated new striated muscle.
Hung *et al [330]*	VF	PLGA	*In vivo*. Cell seeded scaffold implanted subcutaneously in mice	Well-organised neo-fascia formation, traced up to 12 weeks following implantation.

OF. Oral fibroblasts

SIS. Small intestinal submucosa

MDSC. Muscle derived stem cells

BMDSC. Bone marrow derived stem cells

MPEG. methoxy polyethylene glycol

PLA. Polylactic acid

ECM. Extracellular matrix

PLGA. polylactide-co-glycolide

VF. Vaginal fibroblasts

1.10 Background to the project

1.10.1 Formation of matrices – Electrospinning

Electrospinning (Figure 1.10) involves the passage of a polymer solution through a capillary, at which point a high voltage is applied. As the electrostatic forces are overcome at the tip of the capillary, a pendant drop of polymer solution is formed, leading to the ejection of a fine jet of fibre that is attracted towards an earthed collector. During this process, the diameter of the fibre narrows sufficiently for solvent evaporation to occur, which leads to the deposition of polymer on the collector. Typically, this process is driven by various factors including viscosity, humidity, voltage and capillary to collector distance that can influence fibre diameter, pore size and fibre orientation. However, this simple process can be used to reproducibly produce a mat of polymer fibres that can be used as scaffolds for cellular attachment.

A major limitation of this technique involves uncontrollable factors in the methods of the process itself. Humidity and temperature in particular can affect the polymer fibres that are produced, however, these factors can often be controlled to a degree in a laboratory setting.

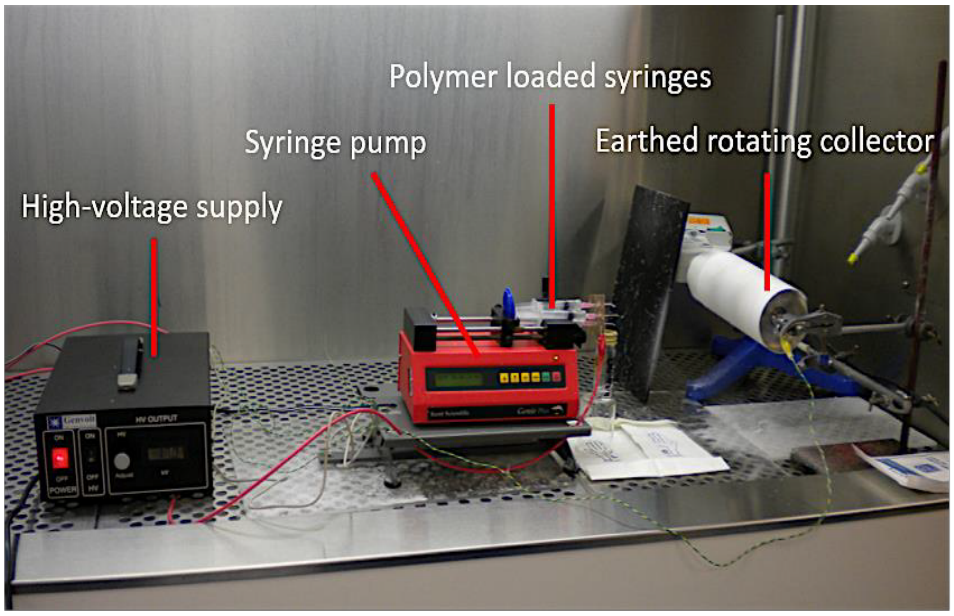


Figure 1.10. ***Electrospinning apparatus***

Charged jet of polymer fibre attracted to earthed collector.

1.10.2 Chemo-stimulation

A variety of pharmacological substances have been investigated to assess whether these confer biomimetic actions upon the cells/host response following implantation. Several key targets are of interest in the regeneration of tissues, particularly the inflammatory response, infection, ECM production (including collagen and elastin) and vasculogenesis. Incorporating such substances into degradable synthetic materials allows a controlled release of the investigated drug corresponding to the degradation of the repair material itself. Due to the controlled production of materials with tuned fibre and pore sizes, not to mention synthetic fibres that can be tuned to degrade rapidly or more slowly, electrospun fibres have been investigated as a vehicle for drug delivery to tissues [339-341]. The high surface to volume ratio of electrospun nanofibre polymers would make these materials ideal vehicles for a drug delivery system by enabling an adequate delivery of drug across a large area. The drawback to the simplest method of electrospinning (the co-electrospinning of solutions of polymer and drug) is that the investigated bioactive substance often undergoes an initial burst release, which may lead to toxic doses in the acute phase. Using electrospun polymer nanofibres that degrade more slowly, a prolonged drug release can be achieved. Unfortunately however, the hydrophobicity of such matrices has the drawback of reduced cell attachment and integration compared to hydrophilic

polymers such as PLA, poly-glycolic acid (PGA) and poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV).

Those rapidly degrading scaffolds, which release drugs over a period of weeks would be ideal for conditions that would not require a drug delivery system to provide mechanical support. In the dynamic environment of the pelvic floor however, biodegradable synthetic materials would be required to persist at the site of implantation for at least a year. The reason for this is not only to provide initial mechanical support for the tissues, but also to enable sufficient tissue regeneration for an appropriate degree of strength to be conferred by the native connective tissues of the pelvic floor. Other techniques using slowly degrading polymer scaffolds utilizing a 'sandwich' fabrication technique with the active ibuprofen drug as the 'filling' produces a relatively rapid diffusion of the drug, while the nanofibre scaffolds persist *in vitro* [342].

1.10.2.1 Antimicrobials and anti-inflammatories

Using a co-electrospinning technique, PLA and Poly (ethylene-co-vinyl-acetate) matrices have been shown to release a controlled dose of the antimicrobial drug, tetracycline [343]. Tetracycline has also been demonstrated to be released from PLGA/polyurethane co-polymers, with the tetracycline itself incorporated into the PLGA fibres only, to correspond with a controlled release [344]. PLA scaffolds laden with the antimicrobial mupirocin has been demonstrated to inhibit *staphylococcus aureus* bacteria, responsible for certain post-operative wound infections [345]. The non-steroidal anti-inflammatory drug ibuprofen has been incorporated into the polymer fibres of PLGA scaffolds and demonstrated a release over a suitable physiological time-frame [346]. In addition to this, the dosages of ibuprofen released were demonstrated to reduce the response of cultured OF to major pro-inflammatory stimulators, without impacting on cell attachment and proliferation. Another non-steroidal anti-inflammatory, meloxicam has been incorporated into electrospun poly vinylacetate (PVA) scaffolds in order to test the transdermal delivery of the drug through snakeskin models [347]. Diclofenac loaded poly-caprolactone scaffolds are purported to prevent abdominal wall adhesion by reducing the inflammatory response [348].

1.10.2.2 Drug releasing scaffolds that improve vasculogenesis

Heparin immobilized electrospun scaffolds that release VEGF have been associated with enhanced endothelialization for potential applications as a vascular graft [349]. Furthermore, Luong-van et al [350] demonstrated retained bioactivity of released heparin after 6 days in the absence of a significant macrophage response to the drug-delivery scaffolds.

1.10.2.3 Oestradiol

Following implantation of any material, an appropriate initial healing phase is required, including host cell integration and neovascularisation. The sex steroid, oestradiol has potent effects on would healing. Oestradiol is an inhibitor of the MMPs that are responsible for collagen degradation [130] and greater tissue strengths resulting from neo-collagen synthesis [113]. Furthermore, patients with SUI who demonstrate atrophic vaginitis following the menopause are treated with topical oestradiol for up to two months, as tissue strength is altered in post- menopausal patients as a result of altered collagen metabolism [132].

Oestrogens play an important physiological role in the cyclical uterine neovascularlation of the female reproductive tract and also in pathological angiogenesis [351]. Oestrogen has been investigated as a therapeutic target due to the pro- angiogenic properties in disease states characterized by poor vascularization in cardiac ischaemia [352] and wound healing. Oestrogen receptor blockage is also a strategy to inhibit pathological neovascularization in breast cancer and diabetic retinopathy. The effects of oestradiol involve the direct stimulation of vascular endothelial cells through the oestrogen receptor [353]. Previously studies have demonstrated that oestradiol can be released from materials to result in beneficial effects on target tissues [354, 355] and drug delivery systems using oestradiol have been used as contraceptive vaginal rings [356] and using electrospinning techniques as coated cardiovascular stents in coronary artery disease [357]. However, to the best of our knowledge the pro-angiogenic property of oestradiol, which is released from a scaffold has not previously been investigated.

1.10.2.4 Vitamin C

Vitamin C is involved in the synthesis of collagen. Collagen molecules undergo post-translational modifications, including hydroxylation of proline and lysyl residues, which is pertinent for the strength and structure of collagen owing to cross-link formation [358]. Vitamin C is a cofactor involved in the activity of both enzymes involved in the hydroxylation of these residues [359]. Studies have demonstrated the benefit of the addition of Vitamin C to media in tissue engineering in order to enhance collagen production [360], whilst type 1 collagen production increased in SIS scaffolds treated with Vitamin C [361].

1.11 The importance of biomechanical properties

Currently, there are no standardized protocols for the measurement of the biomechanical properties of materials prior to implantation. Present data stems from stress-strain curves calculated through uniaxial tensiometry. Furthermore, there is debate as to the exact mechanical forces exhibited in paravaginal tissues of subjects both with and without pelvic floor disorders. A systematic review by Mangera et al in 2011 revealed only one study comparing the mechanical properties of healthy patient's tissues published in the literature [362]. Here, postmenopausal subjects with prolapse demonstrated less elastic tissues, which failed at relatively low levels of strain. Furthermore, increasing age was related to a lower ultimate tensile strength (UTS) [144]. Materials that are therefore too elastic or weak may lead to recurrence, whereas materials that are too rigid may lead to exposure of the repair material. Therefore, an ideal repair material should have mechanical properties comparable to those of healthy paravaginal tissues.

1.12 Previous work at our institution

In 2010, our group investigated 7 different synthetic and biological candidate scaffold materials, one of which was produced in house. Scaffolds were seeded with oral fibroblasts as potential repair materials for the pelvic floor. Of these candidate scaffolds, electrospun Poly-L-lactic acid (PLA) and commercial small intestine submucosa (SIS) performed the best during assessment of cell viability, matrix component production and biomechanical properties [328]. Through this initial work, PLA was selected for further investigation in an attempt to overcome some of the limitations associated with non-biodegradable synthetic materials and biological tissues.

In 2012, PLA scaffolds were seeded with ADSC and implanted into a fascia defect immunocompetent rat model for 7 days prior to material explantation. Samples were tested for histological and immunological outcomes. All cell seeded materials demonstrated evidence of constructive remodeling with demonstration of neovascularization, cell integration and matrix component production [363].

Of materials that release certain drugs, our group developed biodegradable electrospun poly-lactide-co-glycolide scaffolds to release the anti-inflammatory drug ibuprofen [346]. This was demonstrated to release the drug that corresponds with the degradation of the polymer material itself. The ibuprofen attenuated an acute inflammatory response over a seven day period, by which point the scaffold had degraded. There was no significant impact on cell viability or matrix production. This technology is patented by our group for the treatment of chronic wounds.

Chapter II: Materials and Methods

2.1 Scaffolds and cells

2.1.1 Polymer preparation

Three polymers were used during this project, either in isolation or in combination (co-polymer or layers). Poly-L-lactic acid ((PLA) Goodfellow, Cambridge, UK)) at 10% (wt/v) was dissolved in dichloromethane (DCM). Polyurethanes (PU) Z1 and Z3 (Biomer technologies, Cheshire, UK) were dissolved in 50:50 dimethylformide:tetrahydrofuran (DMF:THF) at 6% (wt/v) and 70:30 DMF:THF at 10% (wt/v) respectively. For *in vivo* experiments, commercial PPL mesh (GynecareTM , Johnson & Johnson) was used as supplied. A summary of these polymers is presented in table 2.1.

Table 2.1. *Polymer solutions used during scaffold electrospinning*

Polymer	Source	Solvent	Concentration
Poly-L-lactic acid	Goodfellow	Dichloromethane	10% (wt/v)
Polyurethane Z1A1	Biomer technologies	Dimethylformide/tetrahydrofuran 50:50	6% (wt/v)
Polyurethane Z3A1	Biomer technologies	Dimethylformide/tetrahydrofuran 70:30	10% (wt/v)

2.1.2 Electrospinning

2.1.2.1 Basic electrospinning of random fibres

Polymer solutions (20mls total) were loaded into 5ml syringes fitted with blunt tipped 21G needles (I & J Fisnar, Wayne, New Jersey), placed into a syringe pump (GenieTM Plus, Kent Scientific, USA), and delivered at 40µl/min per syringe during the electrospinning process (figure 1.10). Microfibres were created with an accelerating voltage of 17kV DC from a high voltage supply (Genvolt, UK) and collected on an aluminium foil covered earthed mandrel (80mm diameter, 160mm length) rotating at 300rpm, with a needle to collector distance of 17cm at 21°C and ~30% humidity. Scaffolds were dried at room temperature for 24 hours prior to storage at -20°C.

2.1.2.2 Electrospinning two separate polymers (co-polymers)

Co-polymer scaffolds of Z1:PLA were formed by simultaneously delivering two individual polymer solutions to the collector from polymer delivery equipment placed either side of the mandrel as depicted in Figure 2.1.1. These co-polymers consisted of either 4 syringes of Z1 to 1 syringe of PLA (4:1 Z1 to PLA termed Z1 high (20%) PLA) or 10 syringes of Z1 to 1 syringe of PLA (10:1— Z1 low (9%) PLA).

2.1.2.3 Electrospinning layered polymer scaffolds

Polyurethane layered scaffolds were created by loading solutions of PU Z3 into 5ml syringes fitted with blunt tipped 21G needles, placed into a syringe pump (Genie^{TM}Plus, Kent Scientific, USA). Tri-layers consisted of random-aligned-random orientations (Figure 2.1.2). Random fibres were produced by delivering polymer solutions at a rate of 40µl/min per syringe with an accelerating voltage of 17kV DC from a high voltage supply (Genvolt, UK) and collected on an aluminium foil covered earthed mandrel (80mm diameter, 160mm length) rotating at 300rpm, with a needle to collector distance of 17cm at 21°C and ~30% humidity. Aligned fibres were produced using a voltage of 21kV, a mandrel rotation speed of 6oorpm and a needle to collector distance of 5cm.

Interwoven random-aligned-random fibre morphologies were produced using two separate syringe pumps. Layers were produced using 15mls for each outer randomly orientated fibres, with a 2.5ml overlap for each layer (one syringe pump delivering random fibres, while the other pump delivered aligned fibres) and a 5ml layer of aligned fibres to comprise the central layer.

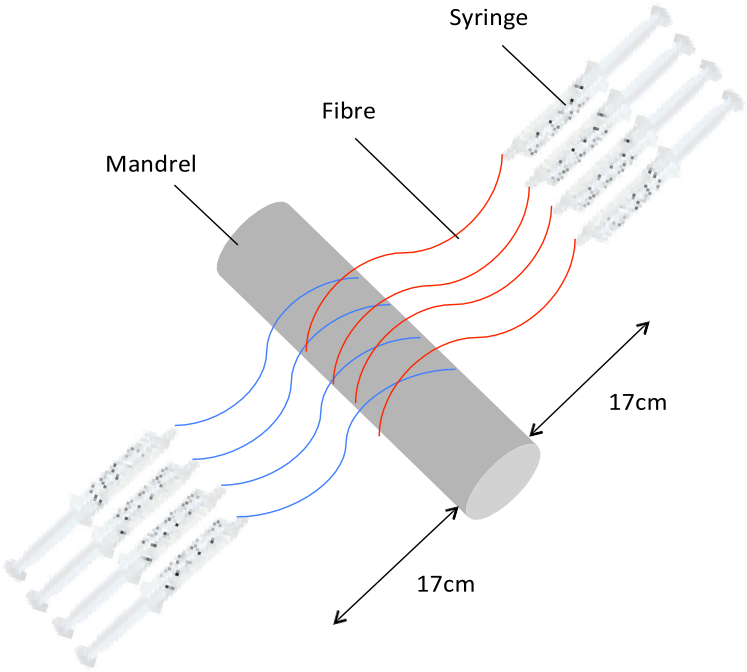


Figure 2.1.1. ***The formation of random electrospun fibres***

Red fibres indicate one polymer solution, while blue fibres indicate another polymer solution.

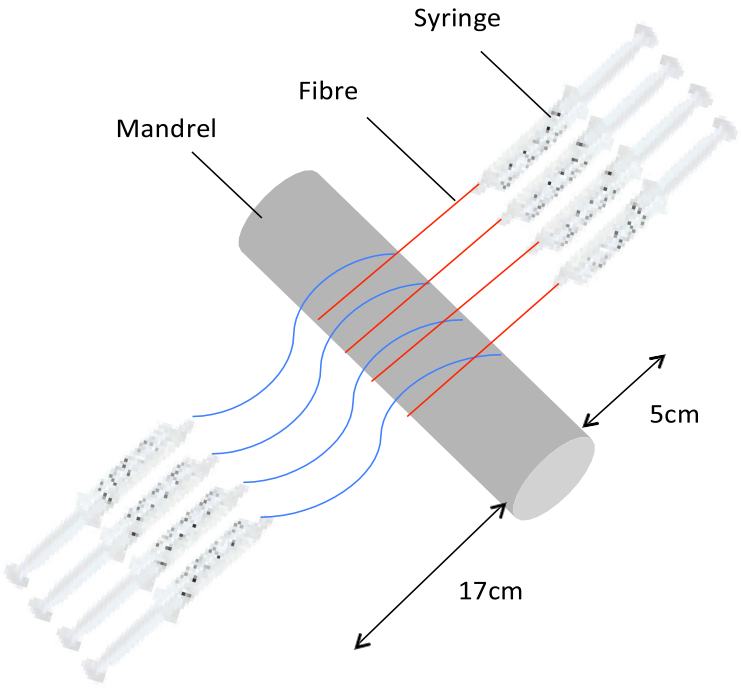


Figure 2.1.2. ***The formation of random-aligned co-polymers***

2.1.3 Isolation and culture of adipose derived stem cells

Human subcutaneous adipose tissue was selected as a source of ADSC. These tissues were obtained from specimens from abdmomioplasty procedures that would otherwise have been discarded following surgery. Procurement was performed on an anonymous basis under research tissue bank license (number 08/H1308/39) under the Human Tissue Authority. All isolation and culture procedures were undertaken in sterile conditions in a culture hood reserved for normal human cell lines. Samples were mechanically minced along with 0.1ml of 100units/ml of penicillin and streptomycin. 10ml of this tissue was collected separately into centrifugation tubes, prewashed with penicillin/streptomycin and centrifuged at 1300RPM for 5 minutes.

The tissue pellet was separated and a mixture of HANK solution, containing 0.1% collagenase A (Roche Diagnostics, Germany), 0.1% bovine fetal albumin (BSA) (Sigma-Aldrich) and 1% penicillin/streptomycin was added. Tissue was incubated at 37°C for 40 minutes with interval agitation. Collagenase digested tissues were centrifuged at 1300RPM for 8 minutes, with the pellet representing the stromal vascular fraction (SVF), which was re-suspended in Dulbecco's modified eagles medium (DMEM). Cells were further centrifuged at 1300RPM for 8 minutes with 20ml of DMEM before the pellet was finally cultured in one T25 flask, incubated at 37°C and 5% CO_2 for 24 hours.

Non-adherent cells were removed with PBS washes and media was changed three times per week. Cells were passaged once at 80-90% confluence using 2ml of trypsin/EDTA (Sigma-Aldrich) with 100,000 cells re-seeded in subsequent T75 flasks. ADSC were characterized using flow cytometry, differentiation assays and antigen staining as previously described by our group and collaborators in Leuven [363-365].

Cells were seeded at between passage 3-6 for all experiments.

2.2 Sample preparation and cell seeding

Scaffolds were chemically sterilized for 15 minutes using 70% ethanol (ETOH), following which, ETOH was removed by washing with excess PBS. Autoclaved 1cm diameter stainless steel cell seeding rings were placed on the surface of the sterilized scaffold. Cells were detached from tissue culture flasks using 2ml of trypsin/EDTA and counted. Passage 3-6 cells were re-suspended in 0.5ml of DMEM and seeded on the scaffold, with a further 2ml of DMEM added to the outside of the ring to maintain the cells in the centre. Constructs were incubated at 37°C at 5% CO_2 for 12 hours before the metal rings were removed using sterile equipment and the scaffolds were transferred to a new 6 well plate with a further 2ml of DMEM added. Samples were incubated with media, which was replaced three times per week.

2.3 Drug releasing scaffolds

2.3.1 Drug releasing scaffolds

17βEstradiol (mW 272.38, Sigma-Aldrich) was dissolved in the PLA solution at 1%, 5% and 10% by mass of polymer (1mg/ml, 5mg/ml, 10mg/ml total). The gradual addition of the drug allowed colourless solutions of each concentration to be produced. The resulting polymer solutions were then electrospun in a random fashion as above. A variety of oestradiol concentrations were used, as it was not possible to calculate the exact proportion of oestradiol that would be A) present in the final scaffold after electrospinning, and B) released from the scaffold over time. It was hypothesized that 5% of the total oestradiol added to the pre-electrospinning solution would be present in the final scaffold; a value that was determined from previous drug-release experiments using hydrophobic drugs [346]. Our previous data demonstrates that PLA degrades over a 6 month period when submerged in a medium, therefore if this final concentration of oestradiol would be released at a steady state, the actual dose delivered would equate to a physiological tissue concentrations of oestradiol. Clearly, the topical oestradiol ointments used in contemporary clinical practice contain supra-physiological concentrations of the drug delivered intermittently to tissues, which avoid toxic effects. Therefore, this lower dose was selected, as this concentration would be delivered continuously to correspond with degradation of the polymer material.

2.3.2 Measurement of oestradiol release

Scaffolds were cut and standardised by mass to equate to 1% of the entire electrospun mat. All scaffolds were washed and incubated in 1ml/well of Phosphate buffered saline (PBS) in a 12-well tissue culture plate. The relative fluorescence of PBS was measured intermittently (Kontron SFM 25 spectralflurometer) at λ_{ex}277nm/λ_{em}310nm, with fresh PBS replaced following each sampling over a 5-month period. Oestradiol concentrations were calculated by preparing standard solutions of known concentration and plotting the relative fluorescence on a standard curve. New standard curves were prepared at each samplingtime-point.

2.3.3 Calculation of viable oestradiol dose

10,000 ADSC were seeded per well on 6 well plates and incubated with DMEM supplemented with concentrations of oestradiol determined from release experiments as above. Cell viability assay using 2.5ml of 5µg/mL of AlamarBlue® in PBS (AbD serotec, Kiddlington, UK) was performed at days 7 and 14.

2.3.4 Cell differentiation assays in 2-D

100,000 human ADSC at passage 3 were seeded per well in a 6 well plate with DMEM. Both oestradiol releasing and non-oestradiol releasing PLA scaffolds (15mm^2 and weight matched) were added to each well, supported by autoclaved stainless steel grids, the surface of which was covered by media. DMEM was removed after 24 hours and replaced with specific induction medium, changed every 3 days for 3 weeks. Adipogenic induction medium consists of 4.5 mg/mL glucose-DMEM (GlutaMaxTM,

Gibco Invitrogen, Paisley, UK) containing 10% (v/v) FBS, 5 mL penicillin (100units/mL) and streptomycin (100 µg/mL), 2.5 ml fungizone (630 ng/mL), l µM dexamethasone (Sigma-Aldrich, Dorset, UK), 0.5 mM methyl-isobutylxanthine (Sigma-Aldrich, Dorset, UK), 10 µg/mL insulin (Sigma-Aldrich, Dorset, UK), and loo µM indomethacin (Sigma-Aldrich, Dorset, UK). Osteogenic induction medium contained 10 nM dexamethasone (Sigma-Aldrich, Dorset, UK), 50 µg/mL ascorbate-2-phosphate (Sigma-Aldrich, Dorset, UK), and 2 mM b-glycerophosphate (Sigma-Aldrich, Dorset, UK) in 4.5 mg/mL glucose-DMEM (GlutaMaxTM, Gibco Invitrogen, Paisley, UK) supplemented with 10% (v/v) FBS, 5 mL penicillin (100units/mL) and streptomycin (100 µg/mL), and 2.5 mL fungizone (630 ng/mL) (Gibco Invitrogen, Paisley, UK).

Cells were fixed with 3.7% (v/v) paraformaldehyde for 20 minutes and washed with PBS. Fixed cells were stained for both adipogenic and osteogenic potential. For adipogenic potential, fixed cells were incubated with 0.3% filtered Oil Red O (Sigma-Aldrich, Dorset, UK) in 60% isopropanol (Fisher Scientific, UK Ltd) (w/v) for 20 minutes. After excess stain was removed by washing with PBS samples were lightly stained with haematoxylin (Sigma-Aldrich, Dorset, UK) and light microscopy was performed. For

osteogenic potential, fixed cells were stained with 1 mg/mL Alizarin RedTM solution (Sigma-Aldrich, Dorset, UK) for 10 min. Light microscopy images were taken after removing excess stain with PBS.

2.4 Measurement of cell response

2.4.1 Metabolic activity

The metabolic response of cells cultured on scaffolds was quantified by AlamarBlue™ (Resazurin) assay at 7 and 14 days. This blue dye becomes irreversibly reduced to the red fluorescent resorufin in the presence of active cells. Media was removed from each well and scaffolds were washed three times in PBS. 1mL per well of sterile resazurin (5mg/ml) was added and cells were incubated for one hour (figure 2.4.1). 50µl of each sample was aspirated with the optical density measured at 570nm using a plate reading colorimeter.

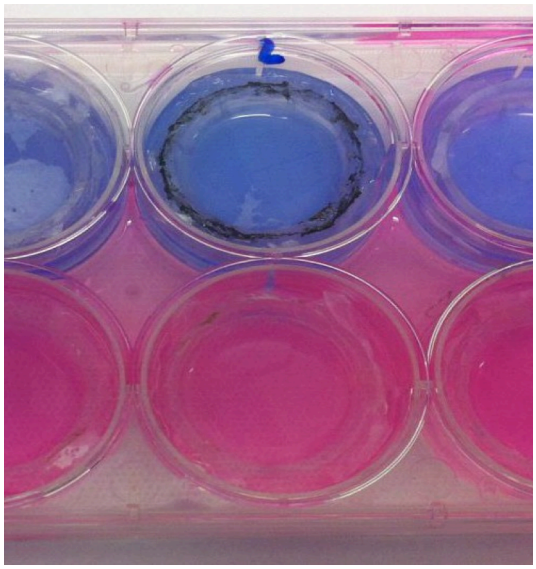


Figure 2.4.1. ***Cell metabolic activity of cultured cells seeded on scaffolds in a well-plate***
Top – non cell seeded scaffolds. Bottom – cell seeded scaffolds. Blue colour indicates no/little activity. Pink colour indicates cell metabolic activity.

2.4.2 Staining for total collagen production

After 14 days, total collagen production was measured of each scaffold. Following three washes with PBS, 1ml of 0.1% solution of Sirius Red F3B in saturated picric acid was added and samples were allowed to stain for 18 hours. Sirius red is a polyazo dye and stains collagen fibres a deep red colour. Samples were washed with PBS until no further stain was eluted. Samples were then weighed and photographed. Stain was eluted using 1ml per well of 0.2M NaOH:MeOH (1:1), allowed to evaporate over 10 minutes. 50µl of each sample was aspirated with the optical density measured at 490nm

using a plate reading colorimeter.

2.4.3 Cell attachment and matrix production –immunofluorescence

500,000 human ADSC at passage 3 were seeded on all scaffolds and cultured for 2 weeks as above. Cells were fixed 3.7% paraformaldehyde for 20 minutes, followed by washing samples 3 times with PBS. 1ml of 1ng/ml of 4′,6-diamidino-2-phenylindole dihydrochloride ((DAPI) Gibco Invitrogen, Paisley, UK) was added to cover each sample for 40 minutes. Samples were washed 3 times in PBS, following which, 1% of bovine serum antigen (BSA) was added for 30 minutes to reduce non-specific binding. Primary antibodies were added; goat anti-human collagen I, III and rabbit anti-human alpha elastin (AbD Serotec, Oxford, UK). 100µl of 1:20 antibody was added per sample for 30 minutes prior to washing with PBS. 100µl of 1:50 secondary antibodies were added, including FITC labelled anti-goat IgG and anti-rabbit IgG.

The location of DAPI stained nuclei and matrix components (fluorescein isothiocyanate (FITC) labelled) were determined using a fluorescent (Axon ImageXpress^{TM}, Molecular Devices Limited, Union City, CA) by switching between filters for DAPI (λ_{ex}385nm/λ_{em}461nm) and FITC (λ_{ex}495nm/λ_{em}515nm).

2.4.4 Examination of cell penetration into scaffolds

For imaging of live cells within scaffolds, live staining was used to label the cells and second harmonic generation (SHG) was used to image the scaffolds. 500,000 ADSC were seeded per scaffold and incubated with DMEM changed three times per week. Cell-scaffolds were cultured for 3 weeks, following which, 0.5mls of serum free DMEM with 10µM celltracker™ red CMTPX (Invitrogen, Oregon USA) was added per well and incubated for one hour. Cells were imaged live, using a Zeiss LSM 510 Meta upright laser-scanning confocal microscope (Carl Zeiss MicroImaging, Germany) using a 40x 1.3 NA oil immersion objective attached to a tuneable (700– 1060 nm) Chameleon Ti:sapphire multiphoton laser (Coherent, CA, USA) for second harmonic generation signal. Red cell tracker signal was created by illuminating constructs at 543nm with 30% transmission and detected between 565nm and 615nm. For SHG signal, constructs were illuminated at 840nm and signals were detected between 415nm and 426nm. Images (512 x 512), with a pixel dwell time of 6.39µs were captured at a range

of depths by moving the focal plane down from the surface of the scaffold, where there was the greatest number of cells present and without any polymer fibres visible, at 1µm intervals until no further cells were visible and polymer fibres dominated the field of view.

2.4.5 Mechanical testing under static and dynamic conditions

Samples of all materials were placed in a tensiometer (BOSE Electroforce instruments, Minnesota, USA) (Figure 2.4.2). Mechanical properties were measured using a ramp test, elongating the material at a rate of 0.1 mm/s or a cyclic test with a rate of 1mm/s up to 25% of displacement from its original length at 50 cycles. Strain was normalized to the sample length, while stress was plotted according to sample area (width x thickness). The point at failure was taken as the UTS and the linear gradient of the initial linear portion of the stress-strain curve was taken as the YM (Figure 2.4.3). Values are expressed as MPa (N/mm^2).

For dynamic loading, samples measuring 3cm x 1cm were placed in a TC-3 load bioreactor (EBERS Medical Technology SL, Zaragoza, Spain) (Figure 2.4.4) and subjected to cyclic uniaxial distension using 25% elongation, 0.1mm/s rate and 18 cycles per minute over 7 days in DMEM at 37°C, 5% CO_2. Samples were then assessed for mechanical properties as above. Data was plotted as stress vs strain and the initial linear gradient of each curve was taken as the Young's modulus (N/mm^2), while the point at maximal stress was taken as the ultimate tensile strength. Both values were compared to values published for paravaginal tissues of healthy premenopausal patients [144]

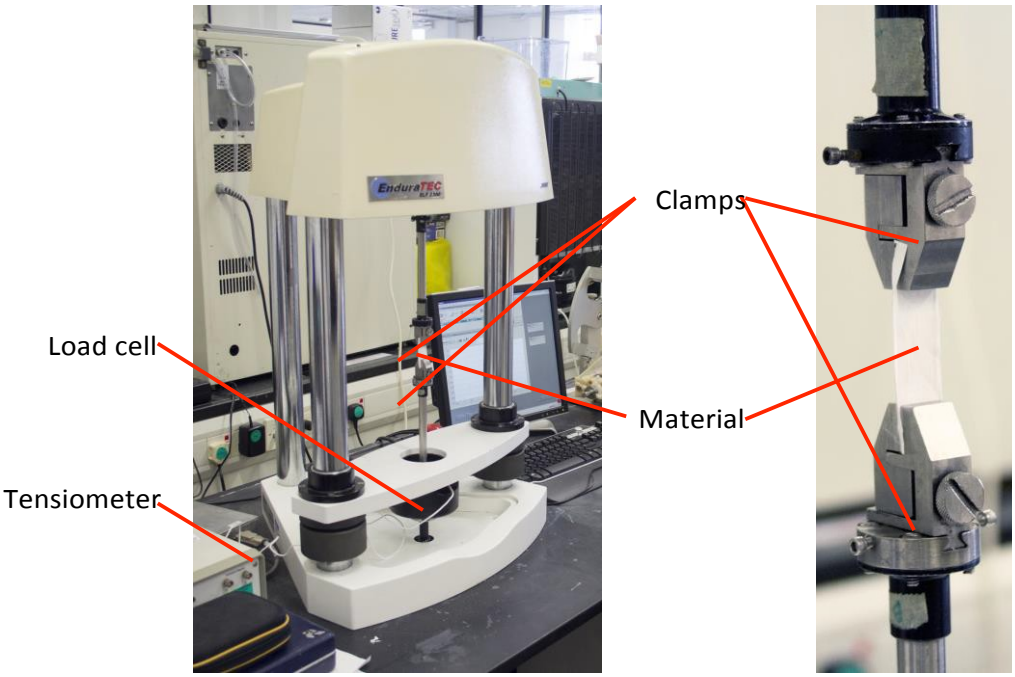


Figure 2.4.2. ***Measuring the tensile properties of tissues/materials using a tensiometer***
Tested material is gripped between two clamps.

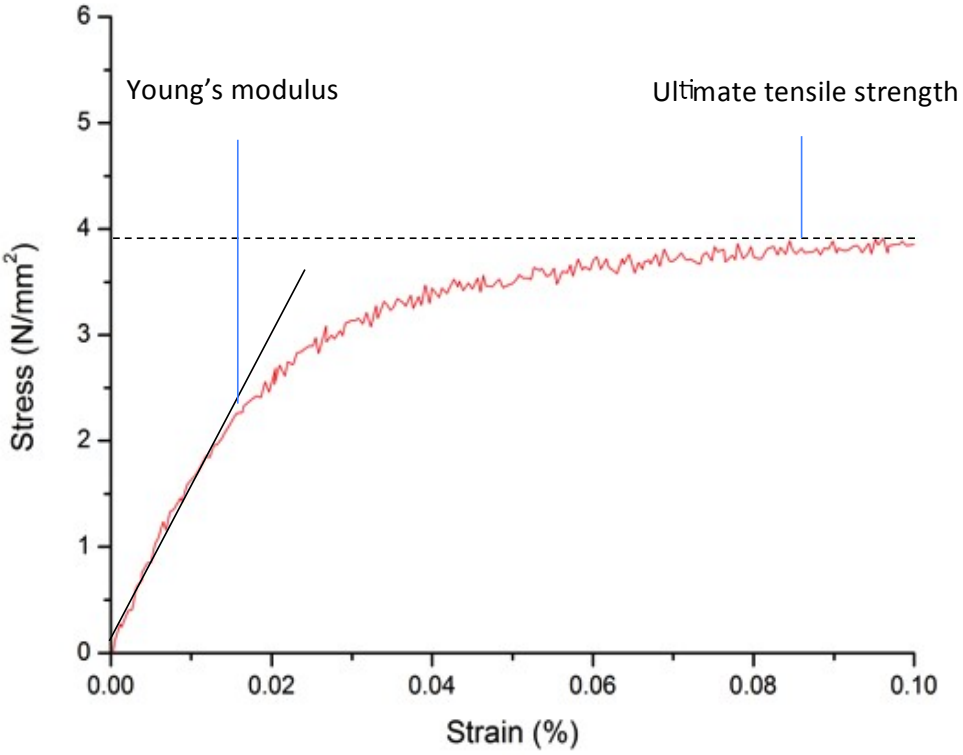


Figure 2.4.3. ***Calculation of Young's modulus and ultimate tensile strength from a stress-strain curve***

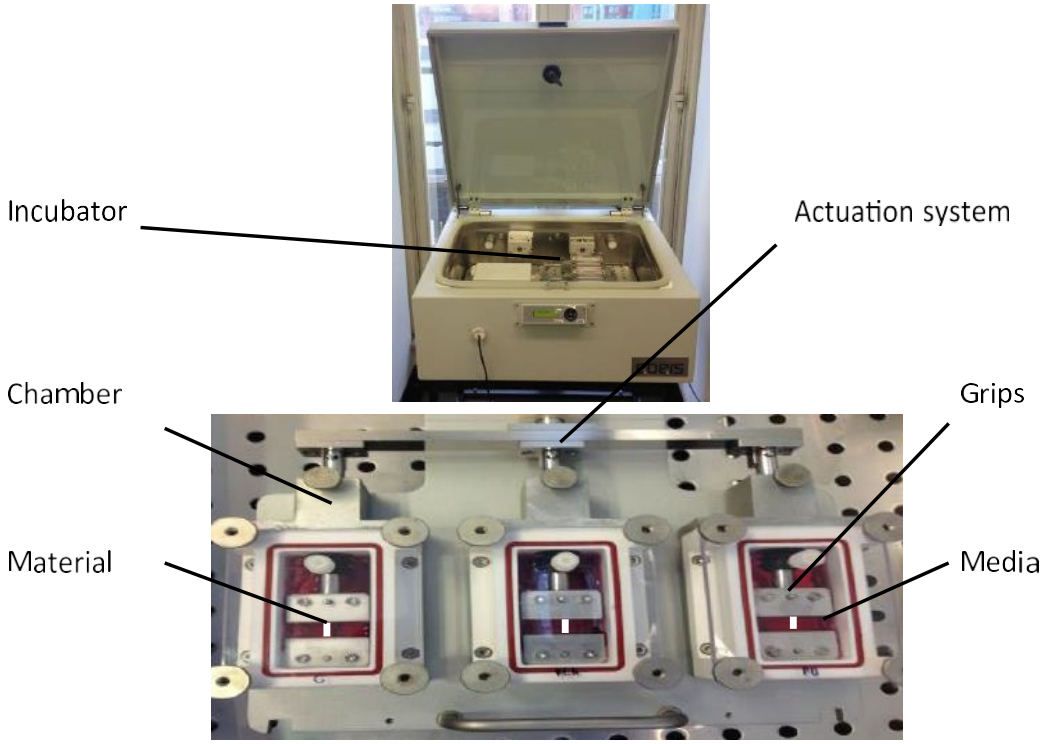


Figure 2.4.4. ***Programmable cyclical distension in a closed incubator bioreactor***

2.4.6 Scanning electron microscopy

Scaffolds were fixed using 3.7% paraformaldehyde and underwent drying using hexamethyldisilazine (HMDS) before mounting on 12.5mm stubs. These samples were sputter coated with approximately 25nm of gold (Edwards sputter coater S150B, Crawley, UK) and examined using a scanning electron microscope (SEM) (Philips/FEI XL-20 SEM, Cambridge, UK) using an accelerating voltage set between 10-15kV.

2.4.7 Statistics

Where P values are given, these were calculated using a two-tailed student T test with equal variance not assumed.

2.5 In vivo examination of scaffolds

2.5.1 Polymers

Electrospun polymer scaffolds included PLA and PU Z3. PPL (Gynecare) and PVDF (DynaMesh) meshes were used as supplied.

2.5.2 Scaffold production

PU Z3 and PLA scaffolds were electrospun and handled in a sterile laminar flow culture hood. PU Z3 solutions (20mls total) were loaded into 5ml syringes fitted with blunt tipped 21G needles, placed into a syringe pump, and delivered at 40µl/min per syringe. Microfibres were created with an accelerating voltage of 17kV DC from a high voltage supply and collected on an aluminium foil covered earthed mandrel (80mm diameter, 160mm length) rotating at 300rpm, with a needle to collector distance of 17cm at 21°C and ~30% humidity. Scaffolds were dried at room temperature for 24 hours prior to storage at -20°C.
(figure 3.1.2).

PLA scaffolds were produced using a novel multi-channel stainless steel delivery tube that was produced in house. The multi-channel tube incorporated 12 delivery needle tips and two tubes were used; each placed either side of the collector attached to separate syringe pumps. One driver delivered random fibres, while the other delivered aligned fibres towards the collecting mandrel. For random and aligned fibers 29 and 15 ml PLA solution, respectively, were electrospun. The multichannel needle was used at 28.8 ml per hour with an accelerating voltage of 17 kV and at 17 cm from the needle tip to collect random fibers, and at 21 kV and 5 cm from the tip of the needles for aligned fibers at 21C and approximately 30% humidity. The collector was set to rotate at 600 rpm for aligned fibers and 300 rpm for random fibers (figure 2.5.1).

Random fibers were electrospun 15 minutes before aligned fibers and finished 15 minutes later. Scaffolds (30 x 30 mm) were cut and packaged in pre-autoclaved bags, which were transported to KU Leuven for implantation.

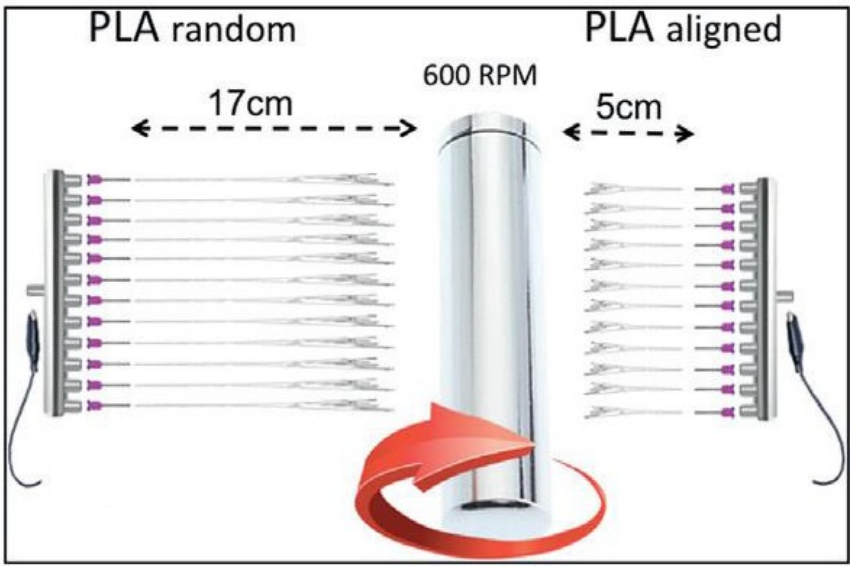


Figure 2.5.1. ***Formation of random-aligned PLA co-polymers using a novel multichannel needle***
Reproduced with permission from Roman, S., I. Urbankova, G. Callewaert, F. Lesage, C. Hillary, N.I. Osman, C.R. Chapple, J. Deprest, and S. MacNeil, Evaluating Alternative Materials for the Treatment of Stress Urinary Incontinence and Pelvic Organ Prolapse: A Comparison of the In Vivo Response to Meshes Implanted in Rabbits. J Urol 2016;196:261-9.

2.5.3 Animals

A total of 40 New Zealand white male rabbits weighing between 3.3 and 4 kg were divided into 5 groups of 8 each according to the implant used during surgery. These included PLA, PU Z3, polyvinylidine fluoride (PVDF) or PPL, with 1 group serving as sham operated controls. Four rabbits per group were sacrificed at 30 days and the remaining 4 per group were sacrificed at 90 days. Rabbits were housed in the KU Leuven animal facility and the experiment was approved by the KU Leuven ethical committee (No. P077-2013). Animals were treated according to the European Legal Framework for the use of animals established by European Union Directive 86/609/EEC. Males animals were selected as subjects, as they are cheaper than females and because they were housed separately, there was no concern over fighting between animals. Clearly, female rabbits would be a more appropriate model in this context, however there are no significant differences in local tissue oestrogens between male and female rabbits before the age of 4 months, furthermore this study assessed the host response to implantation rather than an accurate SUI model. Animal experiments were performed in Leuven, Belgium for two reasons.

1. Our group has a long history of collaboration with researchers in Belgium
2. The Belgian group already held appropriate animal licensing arrangements – procedures which are significantly more difficult to obtain in the contemporary UK climate.

2.5.4 Implantation

Animals were anaesthetized using inhaled 100% isofluorane (Isoba) delivered by a nose cone and the abdomen was shaved. A 60mm longitudinal incision was made superior to the umbilicus and skin flaps either side of the incision were raised (Figure 2.5.2). Two 20 mm incisions were made through fascia and peritoneum in each of the two upper abdominal quadrants, parallel to the midline. Defects were closed under direct vision using 4/0 polyglecaprone (Monocryl) sutures. For each of the 8 sham control defects, no further repair was performed and the superficial tissues were closed using interrupted 4/0 polyglacin (Vicryl) to the dermis and 3/0 Monocryl to the skin. For the experimental arms however, the repaired defect was reinforced with an on-lay of either PLA, PU Z3, PVDF or PPL materials (Figure2.5.3D), secured in a tension free fashion using continuous 4/0 Monocryl suture bites. The orientation of the repair was such that the longitudinal axis of the repair material ran perpendicular to the direction of the fascial incision. Implanted materials were marked with a 4/0 polypropylene (Prolene) suture, placed at each corner to aid in the future identification and orientation of the material. Thereafter, the superficial tissues were closed as previously described (Figure 2.5.3F). Following a period of recover, animals were allowed to move, eat and drink freely. Regular post-operative evaluation on well-being was performed daily for one week and weekly thereafter.

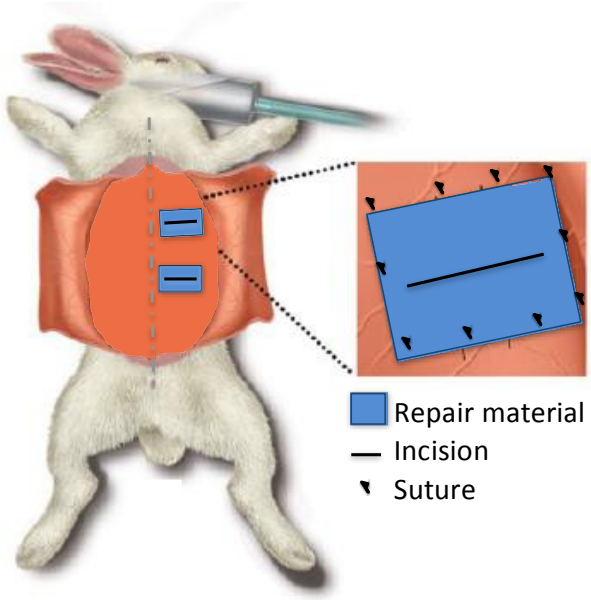


Figure 3.5.2. ***Diagrammatic representation of implantation technique***

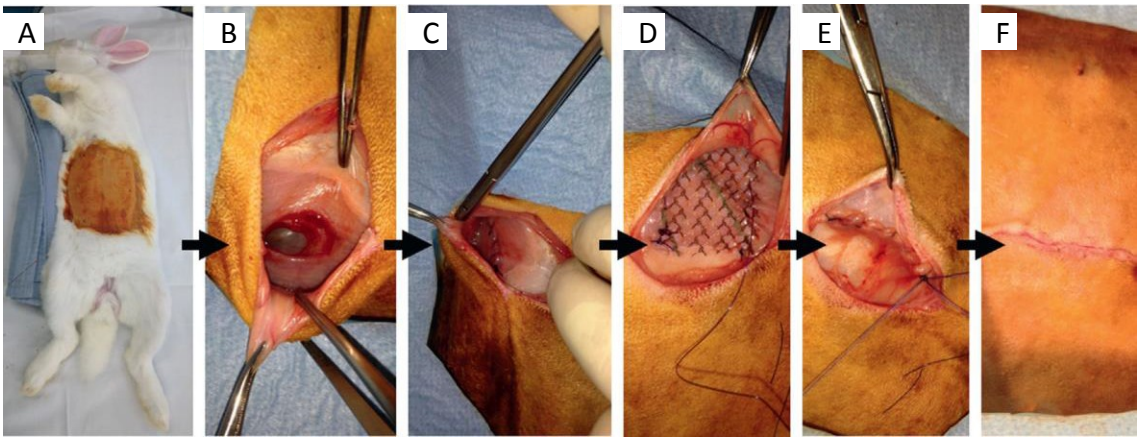


Figure 2.5.3. ***Representative photographs to demonstrate implantation technique***

A) The abdominal wall is shaved and the underlying skin sterilized.

B) A skin incision through the dermis is created and full thickness fascial and peritoneal incisions are created.

C) Fascia and peritoneum are closed.

D) Repair material is sutured to fascia in experimental arms (PPL in this case).

E) Dermis closure.

F) Skin closure.

2.5.5 Animal Sacrifice

At 30 and 90 days following implantation, 4 rabbits per experimental arm were sacrificed at each time point. 1ml of sodium pentobarbital (Nembutal) 60mg/ml intravenously was used following sedation with 1ml each of ketamine and intramuscular xylazine. At the point of explantation, implant dimensions were recorded, in addition to the presence of local complications, which included herniation, adhesion formation, exposure, encapsulation or overt infection. Implanted materials were dissected *en bloc*, to include 15mm of surrounding tissue, termed the explant. Control samples included two healthy samples from virgin abdominal tissue. All explants were divided longitudinally into two separate strips with the defect located at the centre of each strip. One strip of each explant was wrapped in 0.9% saline soaked gauze and stored at -20°C for biomechanical testing. The other strip was fixed in 0.5% zinc chloride, 0.5% zinc acetate and 0.05% calcium acetate in 0.1 M tris HCl, pH 6.4 to 6.7, for 48 hours, following which, it was washed in phosphate buffered saline for 1 hour and transferred to 70% ethanol before paraffin embedding with a Chandon Citadel 1000 HVL device. All samples were transported to the University of Sheffield for further analysis. As discussed preiously, thawing and other post processing techniques do have an effect on the mechanical properties of materials, however this was the only method available for testing these materials; mechanical testing methods were not available in Leuven.

2.5.6 Biomechanical Testing

Explants were thawed at room temperature and cut in a longitudinal fashion. Two samples per explant were produced 20mm x 5mm for mechanical testing. One sample, including surrounding connective tissues was tested, while the second sample was dissected to isolate the implanted material only for testing (Figure 2.5.4). Control (non-implanted) materials that were produced from the same batch were tested. Dimensions were recorded prior to testing using the tensiometer, with an 8mm clamp-to-clamp distance, with the abdominal wall defect located at the centre of each tested sample. Biomechanical properties were tested at room temperature, using a uniaxial tensile test, during which, samples were distended along their longitudinal axis at a rate of 0.1mm/s. Strain was normalized to the sample length, while stress was plotted

according to sample area (width x thickness). The point at failure was taken as the UTS and the linear gradient of the initial linear portion of the stress-strain curve was taken as the YM. Values are expressed as MPa (N/mm^2).

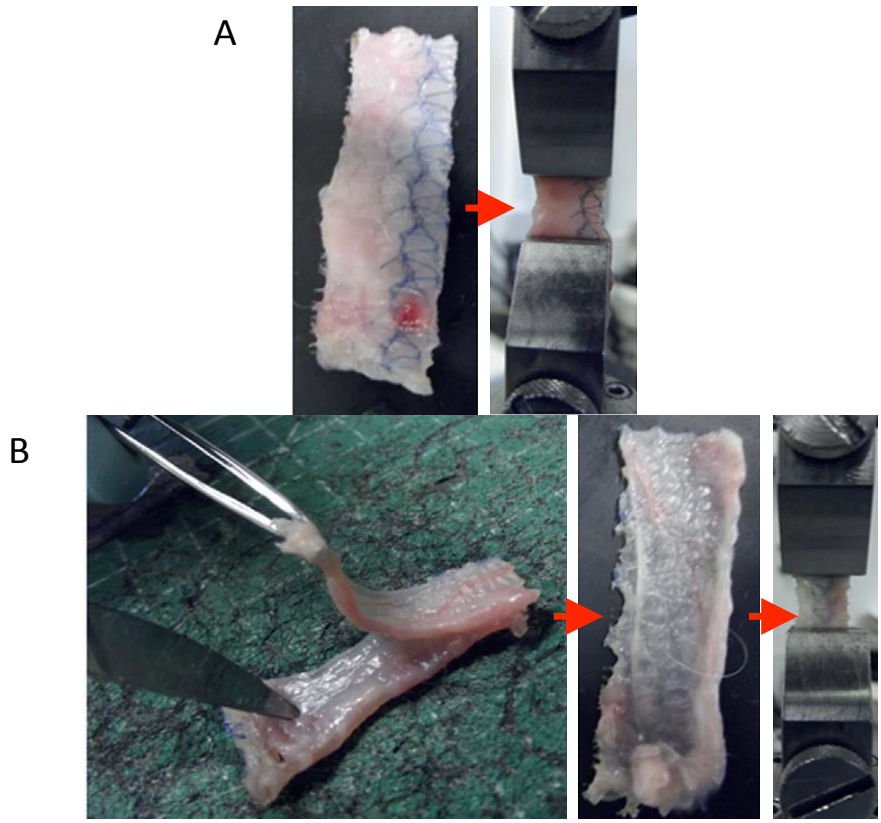


Figure 2.5.4. ***Biomechanical testing of explanted samples***

A) Testing of the material en bloc with surrounding tissues

B) Testing of the material only, following dissection.

2.5.7 Histology

6µm thick sections of paraffin embedded samples was performed using a Leica TP 1020 Automatic Tissue Processor microtome and sections were placed on Superfrost Plus slides (Menzel-Gläser, Denmark). Conventional Haematoxylin and Eosin staining was performed by deparaffinising slides with 100% Xylene (Fisher Scientific, UK Ltd.) for 3 minutes, followed by rehydration of the sample using two submersions in 100% industrial methylated spirit (IMS, Fisher Scientific, UK Ltd.) for 3 minutes each, followed by a further 10 minutes in 95% IMS. Slides were then rinsed with pH neutral distilled water (dH_2O). Slides were then stained with Harris Haematoxylin (Sigma-Aldrich, Dorset, UK) for 8 minutes, followed by 2 minutes of gentle washing with pH neutral dH_2O. Slides were then stained with Eosin (Sigma-Aldrich, Dorset, UK) for 3 minutes followed by sample dehydration by the stepwise submission of slides in 95% IMS (1 minute) and 100% IMS (5 minutes) before cleaning in 70% xylene for 1 minute and mounting using DPX mounting medium (Fisher Scientific, UK Ltd.). Imaging was undertaken using a conventional light microscope.

For immunohistochemistry, 6µm tissue sections were produced and staining was performed using the mouse specific horseradish peroxidase/DAB (diaminobenzidine) (ABC) Detection IHC Kit (Abcam). Sections were rehydrated as above and delineated. A blocking step was performed by incubating samples in 1% BSA (AbD Serotec) for one hour. Sections were then incubated with one of six monoclonal antibodies diluted 1:50 with 1% BSA for one hour each. Table 2.5 provides detail of each primary antibody. Sections were then incubated with biotinylated secondary antibodies, which include anti-mouse Ig using a Detection IHC Kit (Abcam) and anti-goat Ig, in 1% BSA (1:200). After incubation with avidin and biotinylated horseradish peroxidase the target proteins were visualized by incubation in peroxidase substrate and DAB chromogen (Abcam Detection IHC Kit). Samples were counterstained with hematoxylin for 4 minutes, dehydrated and mounted as above.

Control samples consisted of those incubated without primary and secondary antibodies or samples that were incubated only with secondary antibodies. Immunostaining was assessed semi-quantitatively on a blinded observer basis using a qualitative grading scale. Values include absent (0), mild presence (1), large presence (2), abundance (3) and great abundance (4). Three representative images per sample at each time point were evaluated by 4 blinded researchers for a total of 12 samples. Example micrographs that illustrate scores 0, 1, 2, 3 and 4 were provided for reference and the median of these scores was used. The M1/M2 ratio was also calculated for each group.

2.5.8 Statistics

Differences in biomechanical properties between materials and time points were tested for statistical significance by 2-way ANOVA testing. Comparisons between individual groups were determined with the Tukey test. Detecting significance for immunostaining and the M1/M2 ratio were calculated using the Kruskal-Wallis test, while comparisons between individual groups were determined by the Dunn test.

Table 2.5. *Primary antibodies and their description*

Antibody	Description	Rationale	Manufacturer
Mouse anti-human CD31	Surface receptor present on endothelial cells (used to assess neovascularization).	Indicate neovascularisation	DAKO, Agilent Technologies
Mouse anti-rabbit RAM11	Reacts with a cytoplasmic antigen in rabbit macrophages (used to assess the presence of macrophages).	Assess presence of macrophages	DAKO, Agilent Technologies
Mouse anti-rabbit T-Lymphocytes	Clone KEN-5; thymocytes and mesenteric lymph node cells (used to assess the presence of t-lymphocytes).	Assess presence of T-lymphocytes	AbD Serotec
Mouse anti-human HLA-DR	Stains antigen presenting cells such us macrophages, B-cells and dendritic cells. MHC class II cell surface receptor that presents peptides from antigens to T-helper cells which in turn stimulate B-cell proliferation an antibody production. Receptor involved in transplant rejection, several autoimmune conditions and chronic wounds (used to asses M1 response)8.	Assess M1 response	Abcam
Mouse anti-human CD206	Macrophage mannose receptor 1 belonging to the group of pattern recognition receptors, involved in tissue repair (used to asses M2 response)8.	Assess M2 response	AbD Serotec
Goat anti-human collagen III	Extracellular matrix protein	Assess matrix formation	AbD Serotec

Chapter III: The production and characterization of electrospun polymer scaffolds

3.1 Chapter introduction

There is considerable overlap between the techniques of regenerative medicine and tissue engineering. The principles of tissue engineering are based upon the use of scaffolds, which provide an appropriate environment for the penetration and growth of host cells [366]. The term scaffold encompasses a range of synthetic and biological biomaterials and can be pre-seeded with cells prior to implantation or implanted into the body cell-free. The benefit of cell-seeded scaffolds is that the cellular processes of cytokine production, matrix production, angiogenesis and anti-inflammatory effects can optimize the integration of constructs once implanted into the host. Conversely, the implantation of cell-free scaffolds requires appropriate integration of host cells into the scaffolds without the recognized problem of rejection, inflammation or encapsulation. Here, the term scaffold is used to describe synthetic cell-free constructs, and several considerations exist, which dictate the fate of a scaffold following implantation [367]:

1. Scaffold architecture. The material itself, fibre diameter and porosity of the scaffold can affect cell penetration, growth and integration of the cells following implantation.
2. Mechanical properties of the scaffold. Does the strength or elasticity of the scaffold reflect those of the host? Does the scaffold have load-bearing requirements? What will happen to the mechanical properties of the scaffold over time?
3. Host compatibility. Will the scaffold be rejected? Will host cells integrate the material? Will there be encapsulation or an excessive/persistent inflammatory phase?

3.2 Scaffold morphology

Synthetic scaffolds can be composed of either naturally occurring or manufactured polymer materials [368]. The benefit of synthetic scaffolds is that their physical properties can be finely tuned, to achieve appropriate degradation rates, mechanical properties or porosity. They do not usually require any further processing procedures, unlike biological scaffolds and can be produced *en masse* to be available 'off the shelf'.

Electrospinning has become an accepted method for the production of synthetic polymer scaffolds for a host of tissue engineering applications [369]. The production of interwoven fibres creates a 3-D environment with a large surface area for the integration and growth of cells.

In these experiments two classes of polymer are used. The FDA approved polymer, poly-L-lactic acid is a biodegradable polymer of lactic acid, derived from corn starch. Degradation occurs via hydrolysis into lactic acid, which is metabolized via the Krebs cycle. Implanted PLA degrades over a period of months to years, depending on the volume of material that is present. Polyurethanes are a large group of polymers with diverse physical and chemical properties. Polyurethanes (PUs) are degraded by hydrolysis of the urethane bond, with oxidation and enzymatic degradation of the subsequent fragments. However, due to the slow nature of this degradation, most contemporary polyurethanes are considered non-biodegradable [370]. PUs have been used successfully in a host of commercial and medical applications, due to their durability and stability [370]. As a result of these physical characteristics, PUs have been used for insulating cardiac pacing wires, urinary catheters, cardiovascular grafts and breast implants [371]. Degradable isoforms exist, that are less stable yet retain similar physical properties to their non-biodegradable counterparts and have been used in several tissue engineering applications [372].

We investigate both PLA and non-degradable medical grade PUs (PU Z1A1 and PU Z3A1). Scaffolds are produced using a variety of techniques, including electrospinning single polymers, co-polymers or layered polymers:

- Random fibres of PLA (PLA)
- Random fibres of PU Z1A1 (Z1)
- Random fibres of PU Z3A1 (Z3)
- Co-polymer of PLA and Z1 with a high proportion of PLA (Z1 high PLA)
- Co-polymer of PLA and Z1 with a low proportion of PLA (Z1 low PLA)

3.2.1 Fibre morphology

Scanning electron microscopy was performed on all scaffolds to confirm morphology (figure 3.2.1). This allowed the subsequent calculation of fibre diameter and porosity.

3.2.2 Fibre diameter

All scaffolds demonstrated a mean fibre diameter in excess of 0.5µm (figure 3.2.2). Z1 demonstrated the smallest mean fibre diameter of 0.51µm, while PLA had the greatest mean fibre diameter of 0.67µm (table 4.2).

3.2.3 Pore diameter

Using scanning electron microscopy images, pore size was defined as the diameter bordered by overlapping polymer fibres and was imaged using ImageJ software. All scaffolds had a mean pore diameter in excess of 3.5µm (figure 3.2.3), while Z3 demonstrated the greatest mean pore size of 5.8µm. Smaller pores were observed in Z1 (3.9µm) (table 3.2).

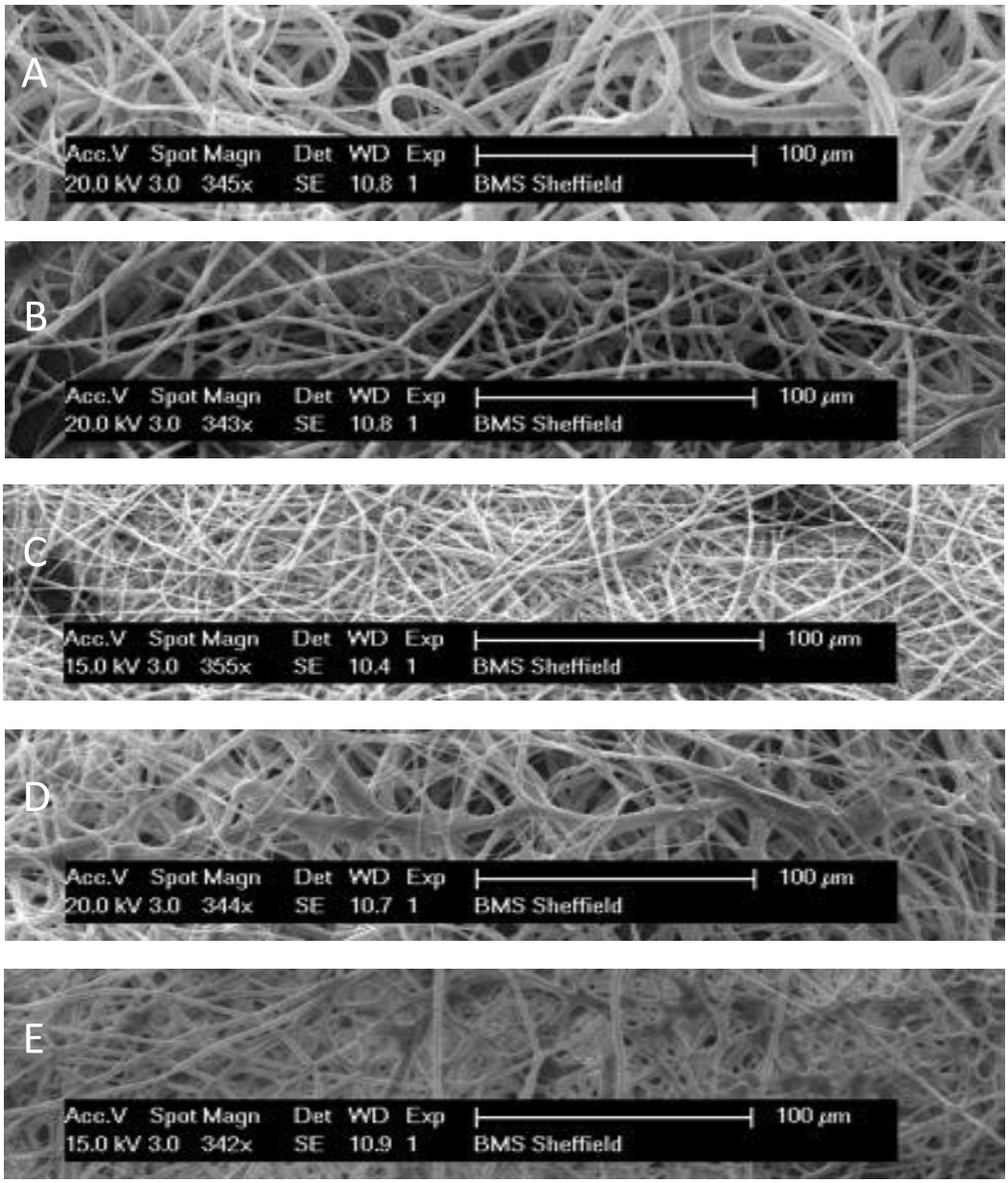


Figure 3.2.1. ***Fibre morphology as demonstrated by scanning electron microscopy***

A) PLA. B) Z1. C) Z3. D) Z1 high PLA. E) Z1 low PLA.

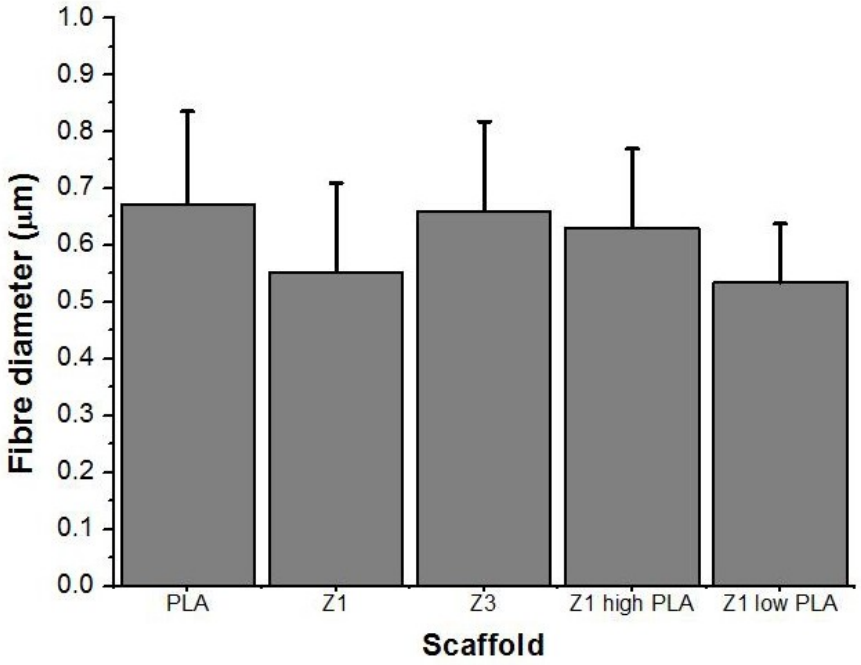


Figure 3.2.2. ***Fibre diameter of scaffolds***

Calculated from serial scanning electron micrographs and ImageJ software. n=20±SEM.

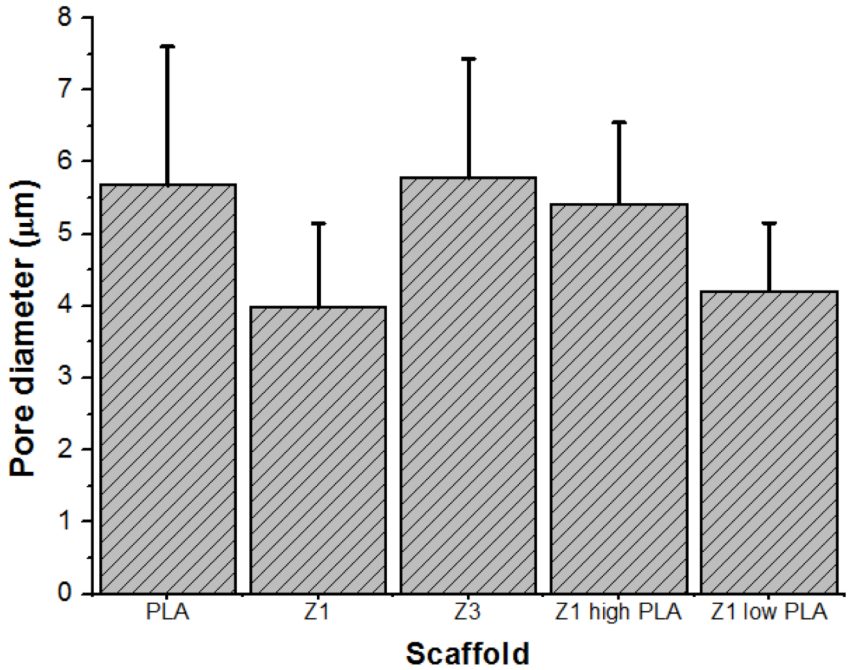


Figure 3.2.3. ***Pore diameter of scaffolds***

Calculated from serial scanning electron micrographs and ImageJ software. n=20±SEM.

Table 3.2. *Summary of scaffold morphological properties*

Scaffold	Fibre diameter (range) (μm)	Pore diameter (range) (μm)
PLA	0.67 (0.49-0.88	5.7 (4.7-7.7)
Z1	0.51 (0.24-0.69)	3.9 (2.1-5.8)
Z3	0.66 (0.48-0.89)	5.8 (4.4-8.1)
Z1 high PLA	0.61 (0.45-0.81)	5.2 (3.9-7.2)
Z1 low PLA	0.52 (0.26-0.71)	4.2 (2.9-5.9)

3.2.4 Section discussion

The structure of any scaffold has a significant impact upon the clinical outcomes that result following implantation. As previously discussed, fibre diameter and pore size can have implications on inflammatory response and implant encapsulation [186]. Therefore, it is important to assess our scaffolds for their morphological properties to ensure that they would be suitable for implantation.

Our produced scaffolds all demonstrate microfibrous, microporous structures; the size of which can be tuned by altering the electrospinning parameters. These include the solution viscosity, the working distance between the syringe and collector, the voltage and rotator speed of the collector [373]. Recent studies have demonstrated a benefit for using microfibrous scaffolds as compared to those composed of much smaller fibres [374, 375].

It is also clear that a relationship exists between fibre diameter and porosity; with greater pore sizes found in scaffolds that exhibit a greater fibre diameter. Pore size is likely to be a more clinically significant morphological component, dictating the ability of cells to penetrate, nutrients to diffuse and macrophages to access the tissues [376]. Some investigators utilize the pore size as an area (μm^2) [377], however, we measure the diameter between fibres, as there is a widely ranging shape between fibres that makes accurate area measurement difficult.

Pore diameter is in excess of 3.5µm; the greater of which are seen in PLA and Z3 scaffolds a relationship exists between the proportion of PLA and greater size. Clearly, these pore sizes would fall into a microporous category (<10µm) based on the Amid classification of surgical mesh. This classification is associated with poorer surgical outcomes, which is attributed to an inability of macrophages and other immune cells to sufficiently integrate the much smaller pores of these materials. Despite this theory, we have demonstrated in several *in vivo* models that both PLA and PU scaffolds are not associated with any significant difference in histological outcomes as compared with polypropylene mesh [363, 378]. This finding may be explained by the electrospun scaffolds demonstrating lightweight and non-

bonded fibres that may exhibit a degree of mobility to facilitate macrophage passage between fibres. While Amid initially described a 75µm pore diameter for macrophages to integrate [186], several authors have demonstrated that this may not be entirely correct [379] and that macrophages are in fact observed in pores of 5µm [380].

Electrospinning as a process is a method that can reliably produce scaffold sheets with tunable morphologies dependent upon the protocols that are used. Clearly, these characteristics will have bearing on the mechanical properties of the scaffold material, the results of which are discussed in the next section.

3.3 Mechanical properties of scaffolds

A uniaxial tensile test was performed for each of the 5 scaffold materials, which allowed the production of a stress-strain curve for each. From the stress-strain curve, the ultimate tensile strength (UTS) and Young's modulus (YM) could be calculated. The values are compared with the range of UTS and YM of healthy (non-POP) tissue taken from the study by Lei *et al* [144], which contains the largest series of living subjects. The intention is to achieve a scaffold that is stronger, yet just as elastic as healthy tissue, without being excessively strong. Commercially available macroporous PPL mesh (Gynemesh, Ethicon, NJ) was also tested in comparison. Dry materials also underwent 10 cycles up to 25% of displacement, with subsequent curves plotted to identify early deformation. Of these experimental materials, PLA, Z1 and Z3 were taken forward to undergo 7 days of continuous distension a bioreactor at 37° and 5% humidity with media at 1mm/s and up to 25% distension, with a repeat tensile test following this.

3.3.1 Tensile test – dry materials

Experimental scaffolds and PPL underwent a ramp tensile test and the stress strain curves both before and after 10 cycles of distension are demonstrated in figure 3.3.1A and figure 3.3.1B respectively. All materials, except for Z1, Z3 and Z1 low PLA fail following 10 cycles of distension (PPL failure indicated by red arrow).

3.3.2 Young's modulus – dry materials

Of the tested materials, only Z3 demonstrates a YM that is in the range observed for healthy tissue (indicated by dotted lines) before 10 cycles of distension (figure 3.3.2A), while PLA has the lowest YM and Z1 high PLA the highest YM before distension. Only Z1 demonstrates no change in the YM following 10 cycles of cyclical distension, while both PPL and PLA become significantly stiffer.

3.3.3 Ultimate tensile strength – dry materials

Of the tested materials, PPL had the highest UTS, while PLA had the lowest figure 3.3.2B. Only Z1 low PLA had a UTS that was in range (indicated by dotted lines) both before and after 10 cycles of distension. Only Z3, PLA and PPL demonstrated an

increase in UTS following 10 cycles of distension, while other scaffolds became weaker.

3.3.4 Plastic deformation

Figure 3.3.3 demonstrates hysteresis loops for each of the dry experimental materials, plotted over cycles 1, 2 and 5. Percentage deformation is calculated as the percentage of change in strain prior to stress at cycle 5. This demonstrates that both Z1, Z3 and Z1 low PLA showed very little change, while Z1 high PLA, PPL and PLA underwent significant deformation.

3.3.5 Effect of dynamic loading under bioreactor conditions

PLA, Z1, Z3 and PPL were assessed before and after 7 days of dynamic distension in a bioreactor. Figure 3.3.4 demonstrates the stress-strain curves before and after 7 days of constant cyclical distension, while figure 3.3.5 shows the YM and UTS as compared to values of healthy tissues (indicated by dotted lines). Only Z1 demonstrated no change in YM and UTS after 7 days under dynamic loading. Z3 became stiffer and stronger, PLA became stiffer and weaker, while PPL became stiffer.

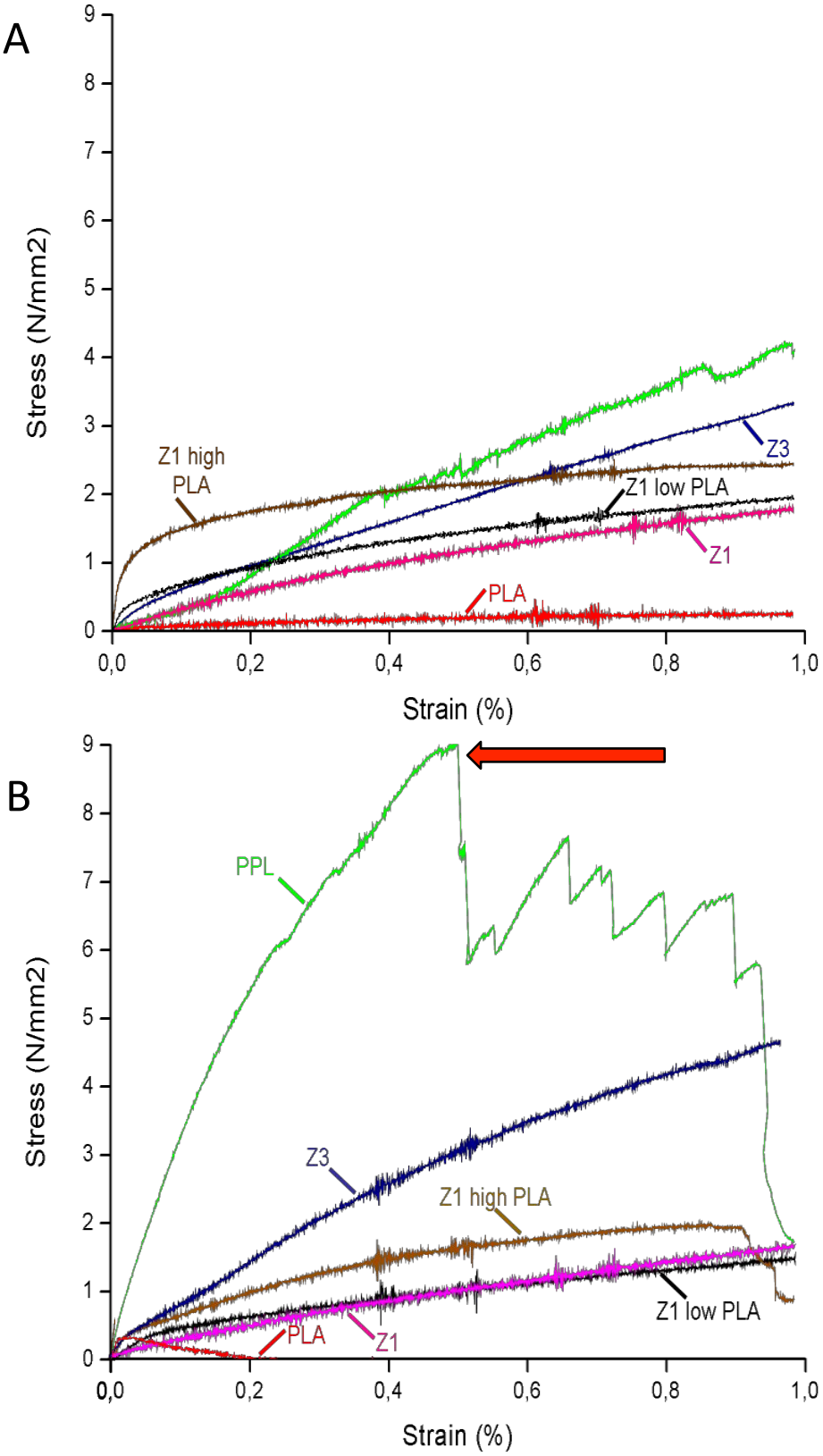


Figure 3.3.1. ***Stress vs strain plots of 6 different materials***

Ramp uniaxial tensile test before (A) and after (B) 10 cycles of distension at 25% displacement.

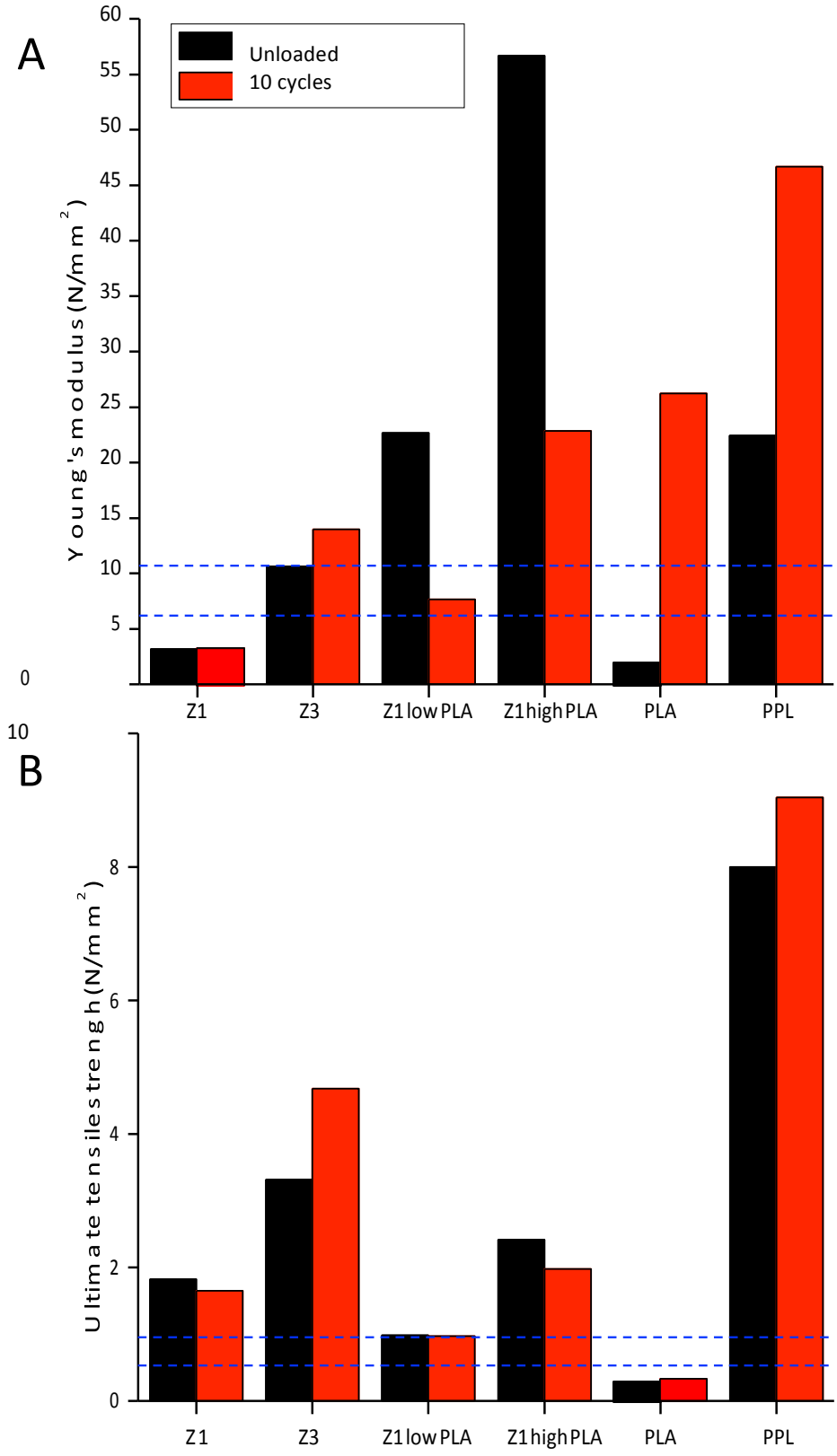


Figure 3.3.2. ***Mechanical properties before and after 10 cycles of deformation*** *Young's modulus (A) and UTS (B) under dry conditions (n=3).*

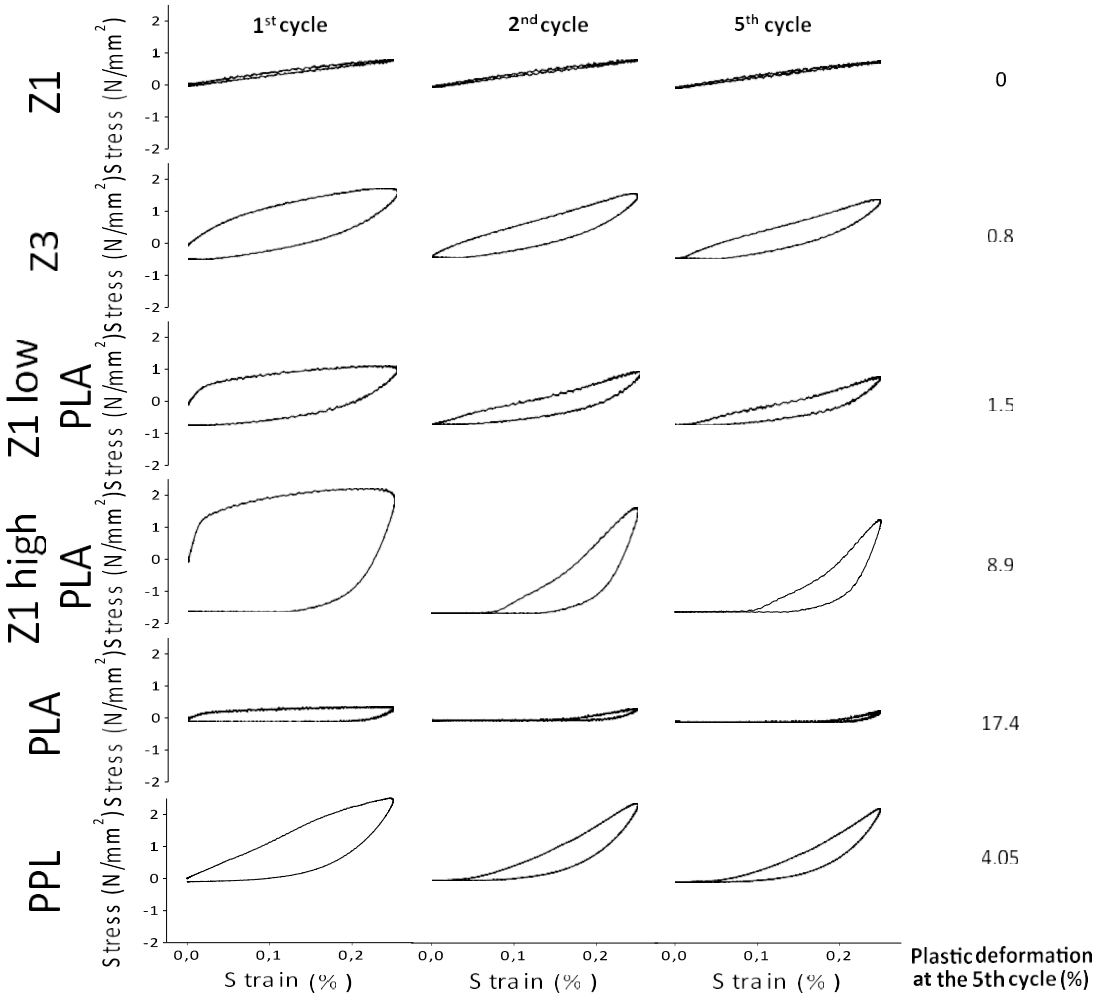


Figure 3.3.3. ***Hysteresis loops of different materials over 5 cycles.***

Plastic deformation (%) at 5th cycle.

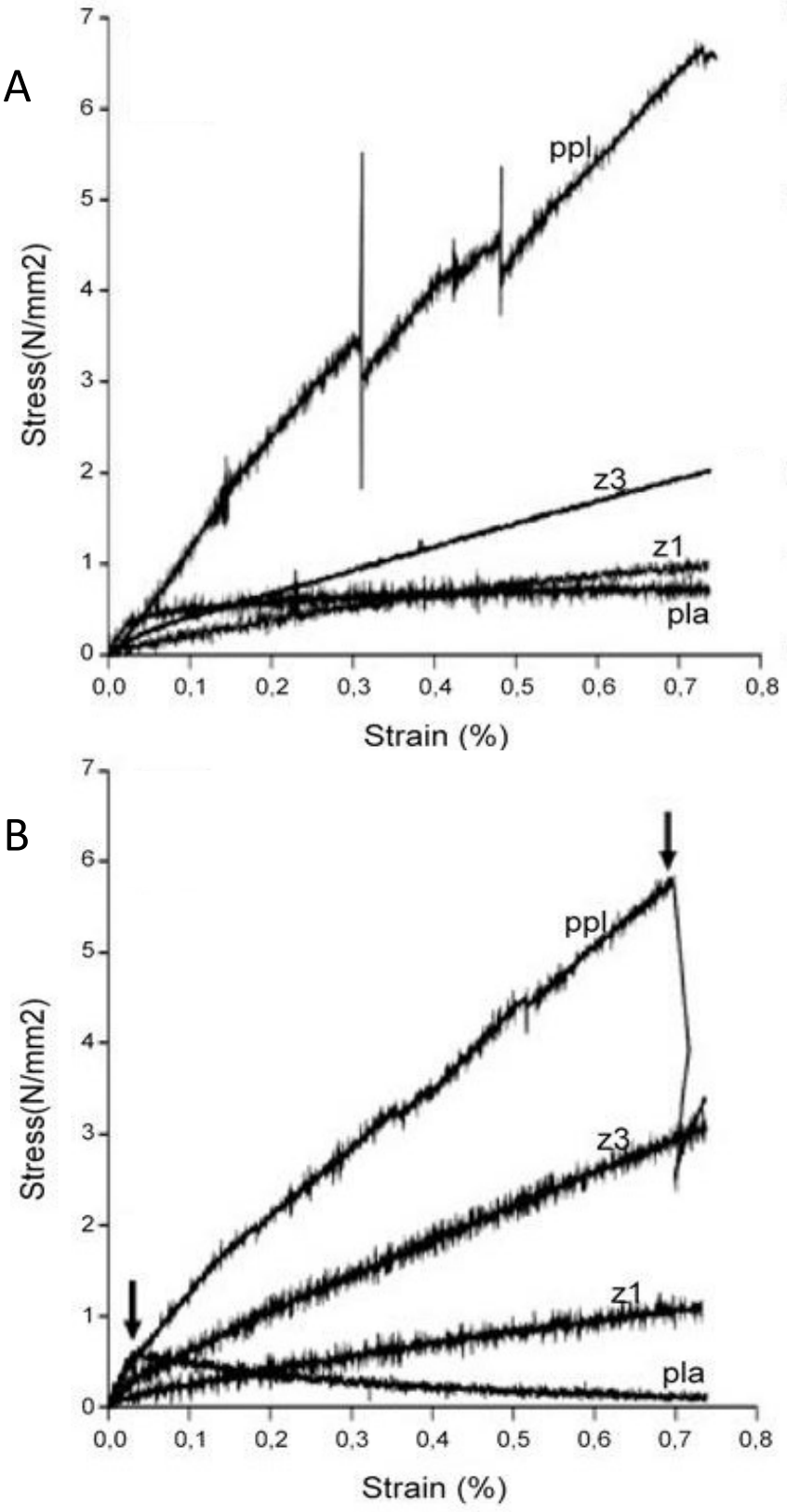


Figure 3.3.4. ***Stress vs strain plots of 4 different materials***

Ramp uniaxial tensile test before (A) and after (B) 7 days of distension in bioreactor. Arrows indicate mechanical failure.

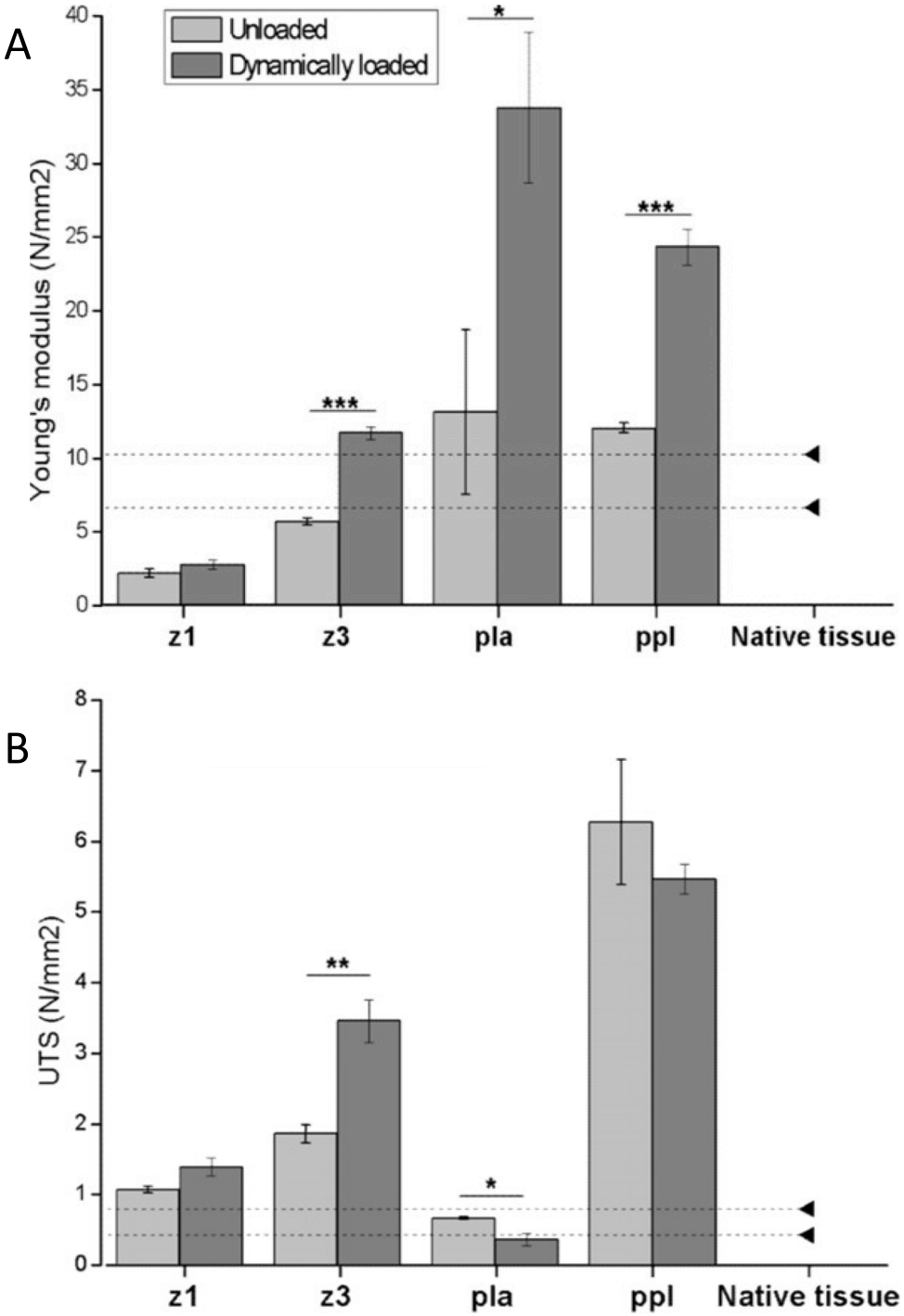


Figure 3.3.5. ***Values for mechanical properties before and after 7 days of dynamic loading*** *Young's modulus (A) and UTS (B) calculated from stress strain curves before and after 7 days of uniaxial distension (n=3±SEM), *p<0.05, **p<0.001. Dotted lines represent values of healthy native tissue.*

3.3.6 Section discussion

The aim is to design synthetic materials, which have mechanical properties that mimic those of healthy fascia; a biomaterial that is associated with high cure rates and few complications when used clinically. Any materials that are designed to serve a load-bearing purpose must possess adequate mechanical properties that fulfill a supportive role of the weakened tissue, in addition to being biocompatible. The implantation of a weak material could lead to recurrence of SUI or POP, while a strong but inelastic material, such as PPL will provide mechanical support but is ultimately incompatible with the pelvic floor environment.

Despite this, no simple correlation exists between the strength of implants and clinical success [362]. It was not appreciated that there are site-specific differences seen with 'soft' tissues. Recent data evidence using sheep models demonstrates that while implanted PPL mesh performs well in the abdominal wall, it undergoes significant contraction and exposure when implanted in the vagina [264]. It has also been shown that although PPL mesh is strong, it is unsuited to dynamic distension with irreversible deformation that occurs during cyclical loading [263].

While there is less concern over biocompatibility with degradable materials, the mechanical deterioration that occurs over time can lead to recurrence of SUI or POP. With non-degradable materials, the concern relates to sustained inflammation that can be associated with complications that develop even several years after implantation [381]. Therefore, biomaterials for the use in pelvic floor reconstruction must not provoke sustained inflammation, while providing appropriate mechanical properties. Therefore, polyurethanes were investigated as candidate materials. Two elastomeric poly-ester-urethranes were selected based upon their viscoelastic properties, Z1 and Z3. Polyurethanes have become popular graft materials in vascular tissue engineering, with Bergmeister [332] demonstrating 100% graft patency rates at one year when cylindrical PU grafts were used as vascular conduits. Meanwhile, Takanari *et al* [333] investigated PU meshes during hernia repair and demonstrated greater elasticity and anti-inflammatory properties resulting with this repair material as compared to conventional PPL mesh.

Using randomly orientated electrospun fibres, several candidate scaffolds were created, with mechanical properties closely related to healthy fascia. PLA has been demonstrated to support cell growth both *in vitro* [382] and *in vivo* [363] and was therefore selected as a candidate scaffold. Co-polymers of polyurethanes with PLA were produced to mimic the mechanical properties of the polyurethane group, while possessing a suitable cell environment that is conferred by the PLA fibres. Despite this, these co-polymers demonstrated significant batch-to-batch variability in their mechanical properties and therefore their investigation was not taken further. Furthermore, we demonstrate that PPL undergoes significant plastic deformation and fails to appropriately recoil following even short periods of distension.

3.4 Cellular response to scaffolds

ADSC were cultured on each of the 5 candidate scaffolds. 70% EToH was used to sterilize all scaffolds. DMEM was changed every 3 days during the incubation protocol. After 14 days, cell-scaffolds were tested for cell viability as measured using AlamarBlue assay and for total collagen production using Sirius red staining protocols. Scanning electron microscopy was used to classify the morphological appearance of matrix surface deposition, while the mechanical properties of scaffolds were retested following two weeks of cell culture to identify mechanical failure. The ability of cells to penetrate scaffolds was tested using confocal microscopy to detect labeled cells at various scaffold depths after 3 weeks of cell culture.

3.4.1 Cell metabolic activity

Cell viability was measured at day 7 and 14 of culture. Absorbance was measured at 570nm using a plate reading colourimeter, minus control scaffolds without cells. Change in absorbance from 7 to 14 days is demonstrated in figure 3.4.1. This demonstrates that all scaffolds showed an increase in cell metabolic activity from day 7 to day 14, however the most significant increase was seen for PLA, Z1 high PLA and Z1 low PLA.

3.4.2 Total collagen production

Total collagen production of ADSC cultured on sterilized scaffolds was tested at day 14 using the methods described for Sirius red assay as above. Values were corrected for weight of scaffolds minus control scaffolds (without cells). These data are presented in figure 3.4.2, which demonstrates a significant increase in total collagen production for PLA, Z1 low PLA and Z1 high PLA.

3.4.3 Matrix deposition on scaffolds

Scaffolds that underwent 2 weeks of culture were fixed and prepared for scanning electron microscopy as previous. These scaffolds demonstrate matrix deposition on the cell surface are shown in figure 3.4.3, showing a dense surface coverage of extracellular matrix over each of the tested scaffolds.

3.4.4 Mechanical properties

All scaffold materials underwent testing using the BOSE electroforce tensiometer before and after two weeks of cell culture. Figure 3.4.4 demonstrates the stress-strain curves for each of the tested material to identify failure. This demonstrates that only the two polyurethanes Z1 and Z3 retain their mechanical properties, while PLA, Z1 low PLA and Z1 high PLA show mechanical failure.

3.4.5 Investigation of cell penetration using fluorescence microscopy

Unfixed cell-scaffold constructs were imaged after 3 weeks of culture at 1µm intervals from the surface (point 0). Red cell-tracker signals were combined with second harmonic generation (SHG) signals for each interval and the results at 4µm intervals are presented in figure 3.4.5. This demonstrates that cells were present within the PLA fibre pores and were able to penetrate this scaffold to the greatest degree of all tested materials, followed by the Z3. Meanwhile, a dense collection of cells was located solely on the surface of Z1 scaffolds without evidence of cells within the polymer pores. For Z1 high PLA and Z1 low PLA scaffolds, cells were able to integrate within the pores but to a lesser degree than PLA scaffolds.

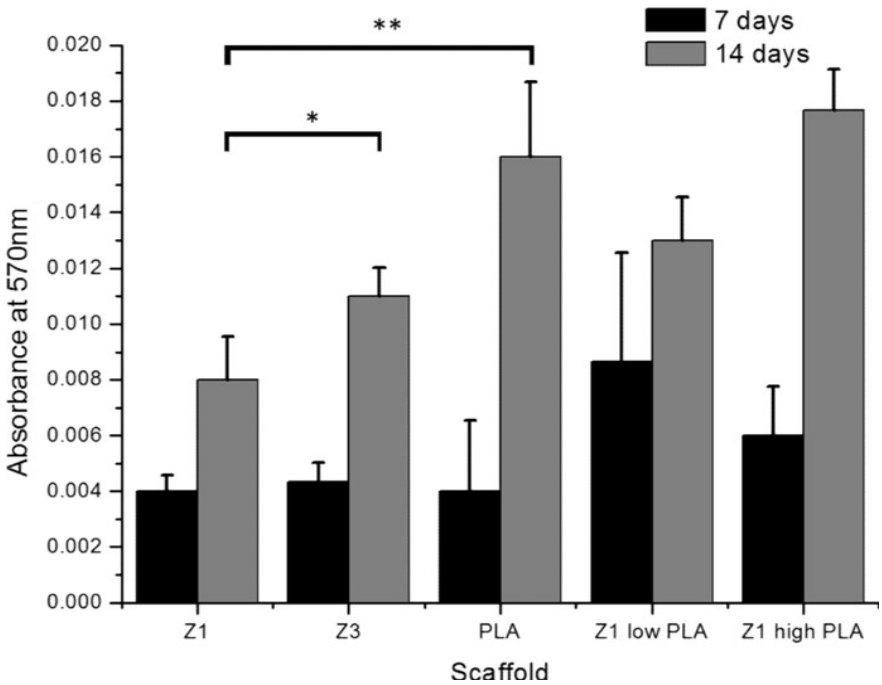


Figure 3.4.1. ***Cell metabolic activity of candidate scaffolds***

*Measured by AlamarBlue assay with absorbance measured at 570nm using plate reading colourimeter. (n=6 ±SEM). *p<0.05 **p<0.01.*

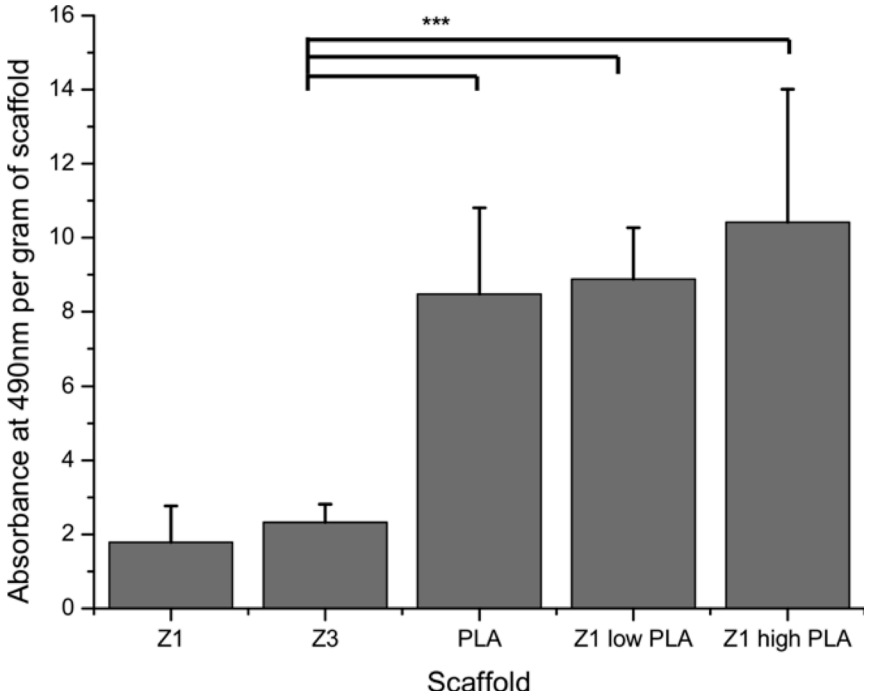


Figure 3.4.2. ***Total collagen production of candidate scaffolds***

*Measured by Sirius red assay with absorbance measured at 490nm using plate reading colourimeter, per gram of scaffold. (n=6±SEM) ***p<0.001.*

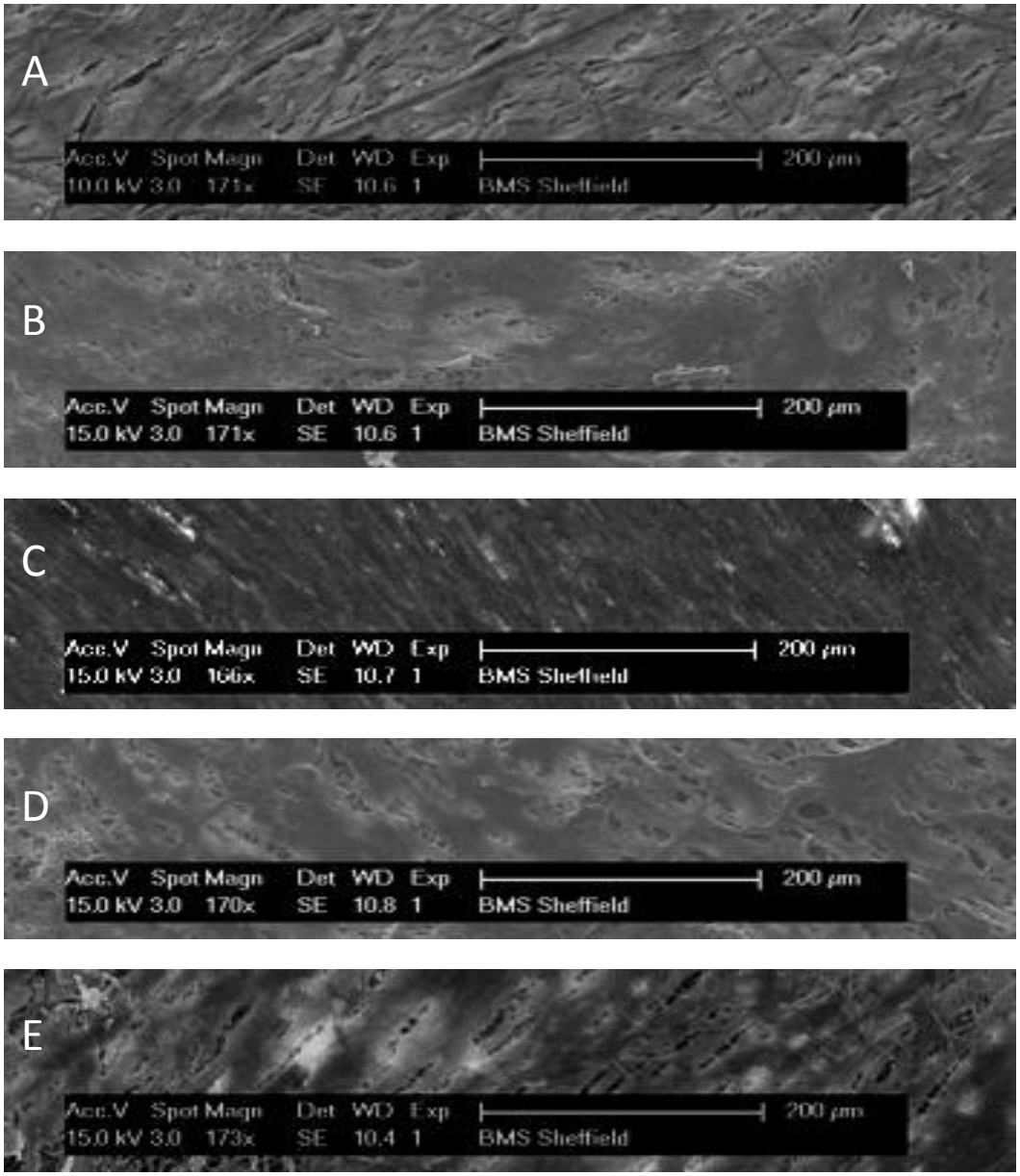


Figure 3.4.3. ***Scanning electron microscopy images of scaffolds following 2 weeks of cell culture to demonstrate matrix deposition on scaffold surface***

A) PLA, B) polyurethane Z1A1, C) polyurethane Z3A1, D) polyurethane Z1A1:PLA (4:1), E) polyurethane Z1A1:PLA (10:1).

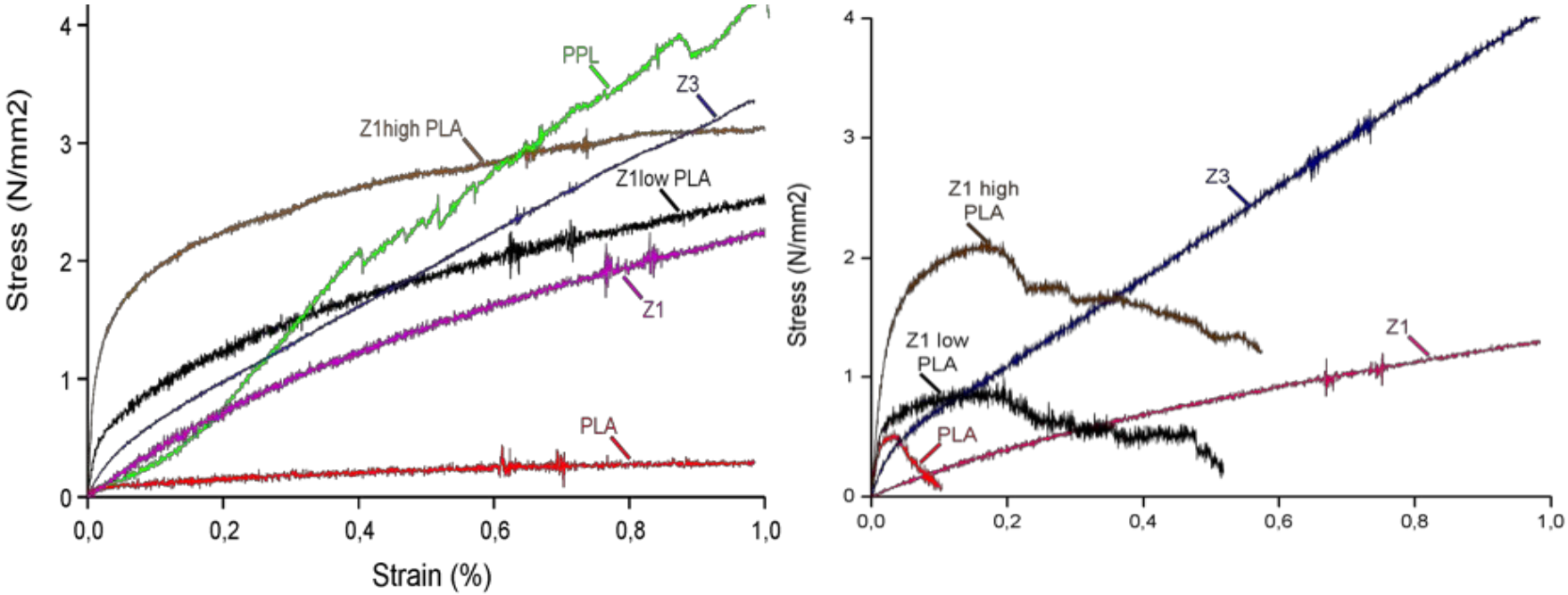


Figure 3.4.4. ***Stress strain curves for 5 materials using the BOSE electroforce tensiometer***

Before (left) and after (right) 2 weeks of cell culture. PPL is not included in cell culture data as the large pore size of this material makes cell culture unfeasible.

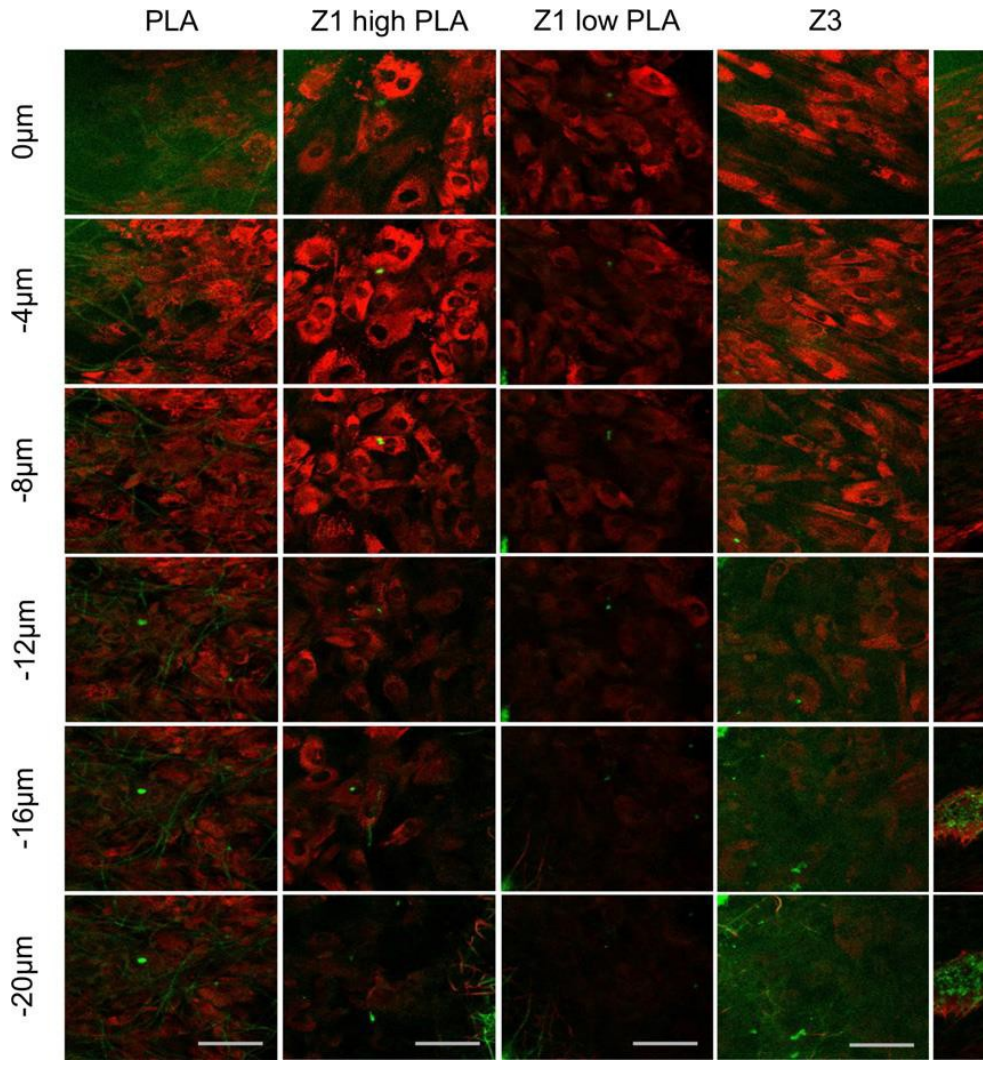


Figure 3.4.5. ***Fluorescence microscopy and scaffold fibre second harmonic generation using microscopy***

Cells (red) and fibre SHG signal (green) for each of the 5 cell-scaffold constructs, imaged from surface (0µm) to 20µm depth. All scale bars equivalent to 50µm.

3.4.6 Section Discussion

Cells that are cultured on scaffolds that contain PLA, show a significantly greater metabolic activity and ability to produce collagen than those cultured on polyurethane scaffolds. Although all tested materials are associated with an increase in viability of cells during the 14 day period of culture, it is likely that the higher porosity of the PLA scaffolds is associated with an increased ability of cells to penetrate the scaffold and therefore the propensity of them to proliferate and produce matrix. This is demonstrated in figure 3.4.5. This cell penetration data shows that even for microporous materials, cells retain their ability to integrate. The concern with microporous repair materials however, is with the ability of macrophages and other inflammatory cells to appropriately penetrate the material in order to resist infection and this issue is tested and discussed in chapter VI.

Human ADSC were used as a cell source for this study as these cells are more proliferative and better defined than fibroblasts and they are capable of contributing to wound healing in several ways making them a popular cell for tissue regeneration [325].

The reasons for mechanical failure of PLA, Z1 low PLA, and Z1 high PLA scaffolds is most likely due to the degradation of these materials that occurs when cultured in media over a 14 day period, and not necessarily as a result of the addition of cells *per se*. In this context, the cell-scaffold complex becomes proportionally weaker despite the increase in total collagen and other matrix proteins that are produced by the cells. Following implantation however, these scaffolds would be expected to become integrated into the host and after a period of remodeling, the strength of the repair site would be conferred by the regenerating tissues rather than the presence of a repair material that will be undergoing degradation.

Z1 and Z3 undergo degradation much more slowly than PLA and therefore it is no surprise that the mechanical properties of these materials remain relatively unchanged following cell culture over 14 days. These polyurethane scaffolds

demonstrate an ability to support cell growth and matrix component production, albeit to a lesser extent than PLA, Z1 low PLA and Z1 high PLA. However, the SEM data does demonstrate the presence of a dense surface matrix covering on both Z1 and Z3 scaffolds, while confocal microscopy shows that cells are able to penetrate Z3 scaffolds.

3.5 Chapter discussion

In these experiments, we investigate the structure and function of a variety of electrospun scaffolds. The primary aim was to assess the ability of these materials to replicate the mechanical properties of healthy fascia and to identify which of these materials possesses the greatest ability to support cell growth, integration and matrix component production in order to identify a leading candidates, which will be implanted in an *in vivo* model.

Ultimately, the mechanical outcomes of these tested materials were modeled on those of healthy paravaginal fascia for two reasons. Firstly, healthy paravaginal tissues are not associated with SUI or POP and secondly, healthy autologous fascia is successfully used for the treatment of SUI as a sling material. The range of mechanical values for healthy fascia are identified and while no single tested material matched the YM and UTS of healthy fascia, Z_3 showed the closest resemblance. Z_3 possessed a UTS that was sufficiently strong, yet not excessively so, a problem that could be associated with a significant discrepancy with native tissues. Furthermore, Z_3 was more elastic than PPL, which enables this material to appropriately stretch and recoil in response to acute distension, without undergoing plastic deformation.

The pore size of PLA is greater than that of the two polyurethanes, Z_1 and Z_3, a finding, which is associated with an increased ability of cells to penetrate this material. As a consequence, cell viability and total collagen production is much greater for cells cultured on this material as compared to any other tested material in these experiments. Despite this finding, PLA becomes weaker during repetitive distension as assessed using an Ebers bioreactor.

The acquisition of this bioreactor model was a key step in the assessment of these scaffold materials, as any implanted material will be subjected to repetitive stress in the body, a factor that has particular importance in the context of pelvic floor conditions, where gravity and sudden repetitive forces can lead to significant strain on tissues [265]. Bioreactor models, such as that used in this study comprises complex components and a multitude of adjustable parameters, but provides an ability to model the basic biological and mechanical processes that occur in the body. The

particular mechanical parameters that were chosen for assessment with the Ebers bioreactor reflect the abdominal wall stretch requirements that are close to 20-30% at maximal forces [383]. However, there is no clear data on the percentage displacement that occurs in pelvic floor tissues. 18 cycles per minute was chosen as an appropriate distension rate to reflect the normal breathing rate of humans. Pelvic floor tissues do not simply undergo distension in a single orientation but are responsive to multiaxial forces. The Ebers bioreactor delivers uniaxial tension to biomaterials in a cyclical fashion and clearly a multiaxial delivery model would be more appropriate, however this would necessitate the use of complex programmable equipment that is beyond the scope of these experiments. Other authors have used the ball burst assessment as a measure of a biomaterial's ability to resist multiaxial distension [384], however these results are often presented as structural strength, which is independent of sample thickness and is therefore an unfair comparison between materials and assumes that a specific material thickness is known.

The exposure of scaffolds to agents during the sterilization process can result in accelerated degradation processes. PLA undergoes degradation through hydrolysis, while polyurethanes predominantly degrade through oxidation. Exposing these polymers to ethanol can therefore accelerate these effects [385], although investigators have demonstrated that the mechanical outcomes of polyurethanes remain relatively unchanged after periods of ethanol exposure [386].

Table 3.5 summarises the key properties of the tested materials. Z3 had mechanical properties that were more closely related to healthy fascia than any other material. By interweaving the fibres of Z1 with PLA, we significantly improved the interaction of cells with the polyurethane material, despite a manifest reduction in elasticity. Meanwhile, PLA demonstrated the greatest ability of tested materials to support cell growth and matrix component production. Therefore, these two materials were selected as candidates to be taken forward for implantation in an abdominal wall defect animal model to assess the *in vivo* outcomes over a 3 month period.

Table 3.5. *Summary of scaffold properties*

Scaffold	Ultimate tensile strength	Rigidity	Response to distension	Cell performance			Overall rank
					Collagen production	Cell penetration	
Z3	++	++	++	+	0	+	**1**
PLA	0	0	0	++	++	++	**2**
Z1 high PLA	0	0	N/A	+	++	+	**3**
Z1	+	+	+	0	0	0	**=4**
Z1 low PLA	0	0	N/A	0	++	+	**=4**
PPL	0	0	0	N/A	N/A	N/A	**5**

Ultimate tensile strength = 0 (<1N/mm² or >5N/mm²), + (1-2N/mm²), ++ (2-4N/mm²).
Rigidity (YM approximation to healthy tissue) = 0 (>200%), + (50-100%), ++ (<50%).
Response to distension (YM approximation to healthy tissue) = 0 (>200%), + (50-100%), ++ (<50%).
Cell viability (increase from 7-14 days) = 0 (<100%), + (100-200%), ++ (>200%).
Collagen production (increase compared to Z1) = 0 (<100%), + (100-200%), ++ (>200%).
Cell penetration (ability of cells to penetrate scaffold pores) = 0 (no penetration), + (cells and fibres present), ++ (cells present within fibre pores)

Chapter IV: Biomimetic scaffolds

4.1 Chapter introduction

The sex steroid, 17-beta-oestradiol is the major oestrogen secreted by premenopausal ovary and it plays a vital role in maintenance of normal structure and function of pelvic tissues. It is an inhibitor of the MMPs that are responsible for collagen degradation [130] and leads to neo-collagen synthesis [113]. Oestradiol directly stimulates vascular endothelial cells through the oestrogen receptor [387] and it has been investigated as a therapeutic target to modify angiogenesis, its pro-angiogenic properties are used in disease states such as cardiac ischaemia [352] and wound healing whereas its blockage is a strategy to inhibit pathological neovascularization in breast cancer and diabetic retinopathy.

The overall aim is to assess the impact that oestradiol has on cell viability and extracellular matrix component production. To achieve this, there are three specific objectives:

- To develop an oestradiol-releasing scaffold that delivers specific concentrations of the drug.
- To assess the morphology of these oestradiol releasing scaffolds.
- To investigate the impact that oestradiol has on extracellular matrix components.

4.2 Structure and function

Oestradiol is poorly soluble in water and readily dissolved in the solvents used for the formation of PLA solutions. These colourless solutions were electrospun in an identical fashion to that used for the formation of other scaffolds. PLA was selected as the drug delivery vehicle, as the degradation rate of this polymer would seem to deliver an appropriate concentration of the drug at an appropriate rate. The concern with the use of any drug delivery system polymer is whether the drug is incorporated into the polymer itself or is it present outside of the polymer. I.e, in the context of polymer microfibres, is the oestradiol present within the fibres, on the surface or in the pores?

Ideally, for controlled release, the drug should be present within the polymer fibres, with release of oestradiol corresponding with degradation of the polymer itself. If the drug is present solely on the surface of the construct or within the pores of the scaffold, then an initial burst release of the drug will occur, with little if any further release occurring over time. To test this, release of the drug into a solvent (PBS) was measured at set intervals, with new solvent added after each time point. Furthermore, the addition of any further variation in the electrospinning parameters or composition of polymer solutions can affect the morphology of the produced scaffold [347] and therefore an assessment of morphology, fibre diameter and pore size were performed.

4.2.1 Fibre morphology

Scanning electron microscopy was performed on both oestradiol releasing and non-oestradiol releasing PLA scaffolds to confirm morphology (figure 4.2.1). The addition of oestradiol into the electrospinning parameters did not impact upon the microfibre appearances of the scaffolds under SEM. These micrographs also allowed the subsequent calculation of fibre diameter and porosity.

4.2.2 Fibre size and porosity

Figure 4.2.2 demonstrates the fibre diameter and pore size as calculated from serial scanning electron micrographs. This demonstrates that there is no significant

difference in fibre diameter or pore size between the oestradiol and non-oestradiol releasing PLA scaffolds (0.71±0.28µm vs 0.65±0.30µm [p=0.47] and 4.25 ±2.04µm vs 4.26±2.29µm [p= 0.98] respectively).

4.2.3 Oestradiol release

Release of oestradiol from PLA scaffolds into PBS media was measured fluorimetrically at set intervals over a 5-month period, with the concentration of oestradiol calculated using a standard curve. The cumulative release of oestradiol increased for each time-point, until no further oestradiol was released (at 133 days) as demonstrated in Figure 4.2.3. The total released oestradiol from the scaffolds was equivalent to 2.5%, 1.4% and 2.45% of the oestradiol present in the polymer solution for the 10mg, 50mg and 100mg oestradiol scaffolds respectively prior to the electrospinning process, while 40%, 50% and 40% of oestradiol was released over the initial 14 days for each of the 3 scaffold groups respectively; the rate of release was proportional to the amount of oestradiol present in the scaffold at commencement and the rate reduced overtime.

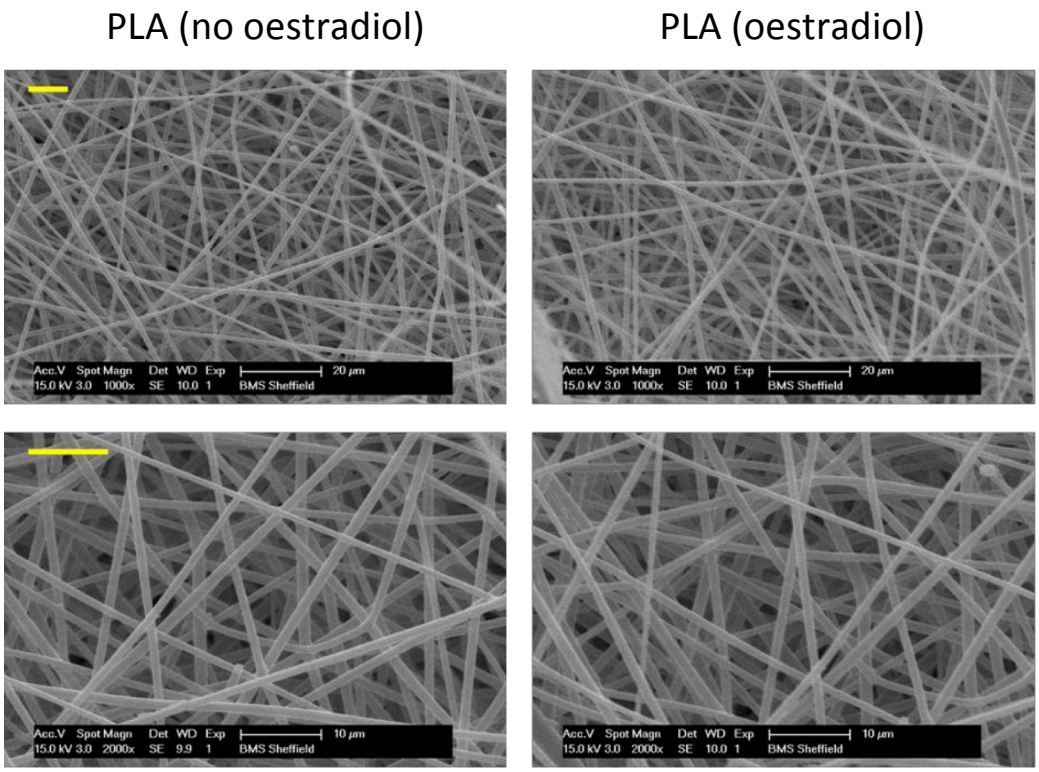


Figure 4.2.1. ***Scanning electron microscopy images of oestradiol and non-oestradiol releasing scaffolds*** *(Top) low magnification, (bottom) high magnification. All scale bars equivalent to 10µm.*

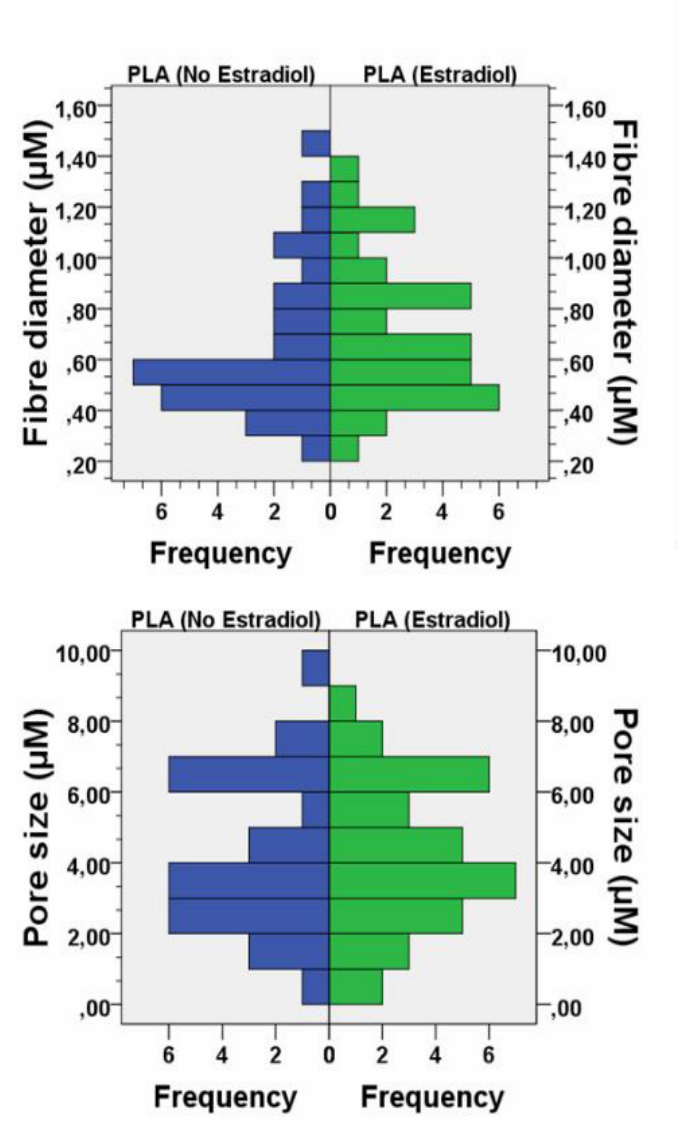


Figure 4.2.2. ***Calculation of fibre diameter and pore size***

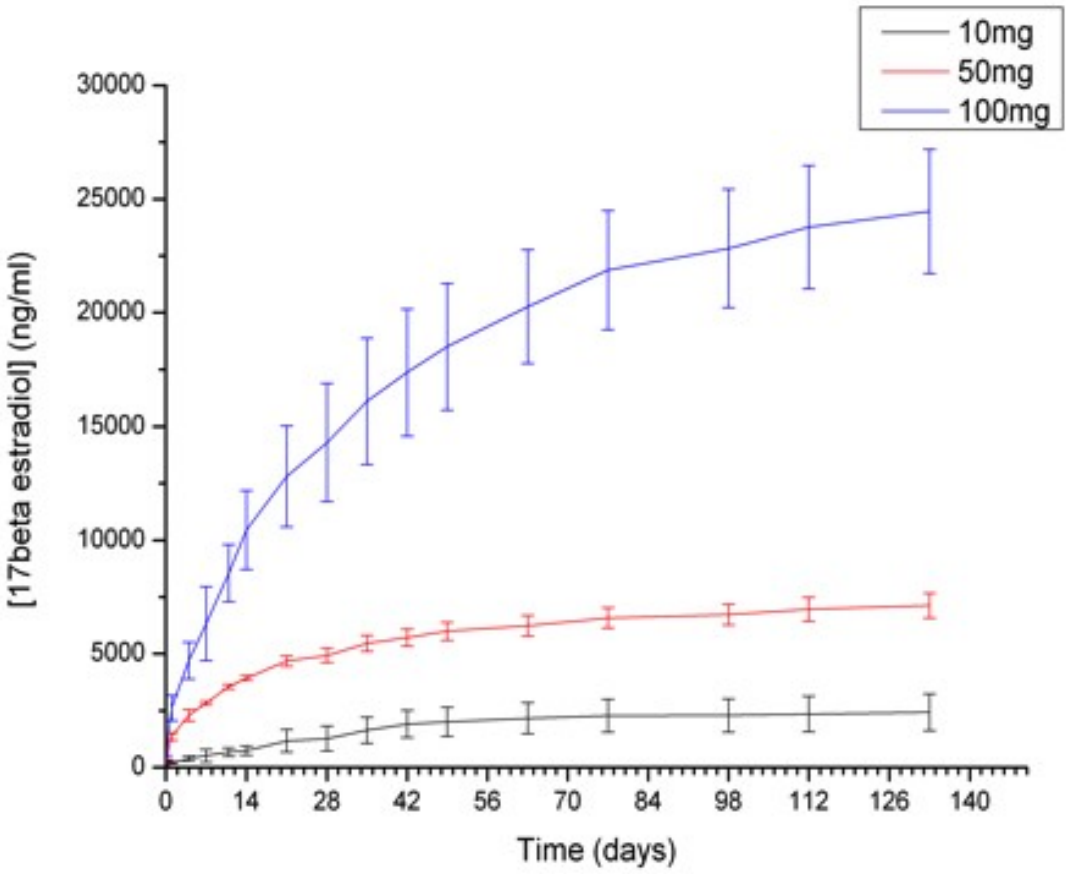


Figure 4.2.3. ***Release of oestradiol from PLA scaffold*** *measured using a spectralfluorometer (n=6 ±SEM).*

4.2.4 Section discussion

Oestradiol was successfully incorporated into PLA microfibre scaffolds with a resulting macroscopic appearance that was similar to that of non-oestradiol containing PLA scaffolds. SEM analysis demonstrates that the ultrastructural appearances of each scaffold were identical, with no significant differences in fibre diameter or pore size.

Oestradiol release was calculated fluorimetrically, the exact concentration of which was measured against pre-prepared standards of known concentration. The exact concentration released by each of the 3 different oestradiol-releasing scaffolds was below that of the physiological serum oestradiol concention (10^{-8}M) and above known effective oestradiol concentrations (10^{-10}M). Therefore, potential toxicity effects are unlikely. The surprising finding is that the actual concentration of oestradiol released is only 2% of that which was prepared in the initial drug-polymer concentration. This highlights the sheer volume of solvent that is lost to the surroundings during the electrospinning process. The exact proportion of drug that is lost during this process and is actually present in the scaffold is difficult to calculate, as oestradiol is poorly soluble in water and conventional solvents interfere with the measurement of the drug using spectroscopy or other biochemical methods. Nevertheless, at low concentrations, the accurate measurement of oestradiol can be performed using water as the solvent.

There is a significant increase in the concentration of oestradiol that is released from the 50mg oestradiol scaffold to the 100mg material. The amount of released oestradiol rises exponentially with the concentration that is initially present in a dose dependent fashion. Furthermore, it is likely that the presence of oestradiol in scaffold fibres causes them to degrade much more rapidly, which is reflected in the exponential rise in released oestradiol.

The rate of release can be affected by altering a variety of variables. The polymer that is used was selected for its predictable and slow degradation. More rapidly degrading polymers, such as polyglycolic acid (PGA) or polyhydroxybutyric acid (PHBV) would

degrade much faster and therefore result in a rapid release of oestradiol over weeks, while polycaprelactone (PCL) would degrade slower.

Between each sampling intervals, the solvent (PBS) was discarded, with fresh solvent added following each measurement. This avoids the formation of precipitates and the disruption of drug release that can occur at high solute concentrations. The accurate fluoroscopic measurement of oestradiol concentration was achieved with the production of fresh standard concentrations at each time point and regular calibration of the equipment.

4.3 Cellular and mechanical outcomes

The PLA scaffold that contained a 5% w/v concentration of oestradiol was selected for further assessment. Cell morphology assays were performed to ensure cultured cells did not preferentially differentiate towards a fat or bony lineage in response to oestradiol. Viability assays and matrix component production was subsequently performed on cells cultured with these scaffolds, in addition to an assessment of the mechanical properties of the oestradiol releasing constructs.

4.3.1 Assessment of cellular differentiation in response to oestradiol

ADSC were cultured in the presence of these scaffolds and appropriate differentiation media for 3 weeks to give an opportunity for the ADSC to differentiate into their well-recognised phenotypes and to assess whether the release of oestradiol affected this.

There were no significant histological differences of cells cultured in DMEM in the presence of either control scaffolds or oestradiol releasing PLA scaffolds, when stained with either Oil red O or Alizarin red, as demonstrated in figure 4.3.1. Culturing ADSC in specific induction media resulted in cellular differentiation over 3 weeks, including the formation of lipidic vesicles for ADSC cultured in adipogenic medium and the presence of calcium for cells cultured in osteogenic medium. However, there were no significant differences in cell morphology, lipidic vesicle or calcium content between control and oestradiol-releasing scaffolds, as assessed using blind scoring.

4.3.2 Cell metabolic activity

Any scaffolds implanted in the body will become populated with cells. In this study, we used adipose derived MSCs as a representative cell population to assess the response of cells to the oestradiol loaded scaffolds.

Figure 4.3.2 demonstrates an increase in cell metabolic activity from 7 to 14 days for cells cultured on both PLA scaffolds and those that release oestradiol. There is, however a significantly greater increase in activity at day 14 for cells cultured on scaffolds that release oestradiol ($P<0.01$).

4.3.3 Total collagen production

Adipose derived stem cells cultured on PLA scaffolds that released oestradiol for 14 days produced a greater proportion of collagen than scaffolds that did not release oestradiol ($P<0.001$) as demonstrated in figure 4.3.3.

4.3.4 Matrix component production

Figure 4.3.4 demonstrates the presence of a greater proportion of collagen types I and III and elastin resulting from culture of ADSC on PLA scaffolds that release oestradiol than from scaffolds that do not.

4.3.5 Mechanical properties

Figure 4.3.5A demonstrates the Young's modulus of oestradiol and control scaffolds both before and after culture with ADSC. After 2 weeks of cell culture, both scaffolds become more elastic, however PLA scaffolds that release oestradiol are significantly stiffer ($P<0.01$). Oestradiol scaffolds demonstrate a Young's Modulus that is closer to the values of healthy native fascia.

Figure 4.3.5B demonstrates that PLA scaffolds that release oestradiol are significantly stronger than those that do not release oestradiol, both before and after 2 weeks of cell culture. Of note, the UTS of control scaffolds decreases after 14 days of cell culture, while the UTS of oestradiol releasing scaffolds increases over this time. Furthermore, PLA scaffolds that release oestradiol have a higher UTS than healthy native fascia.

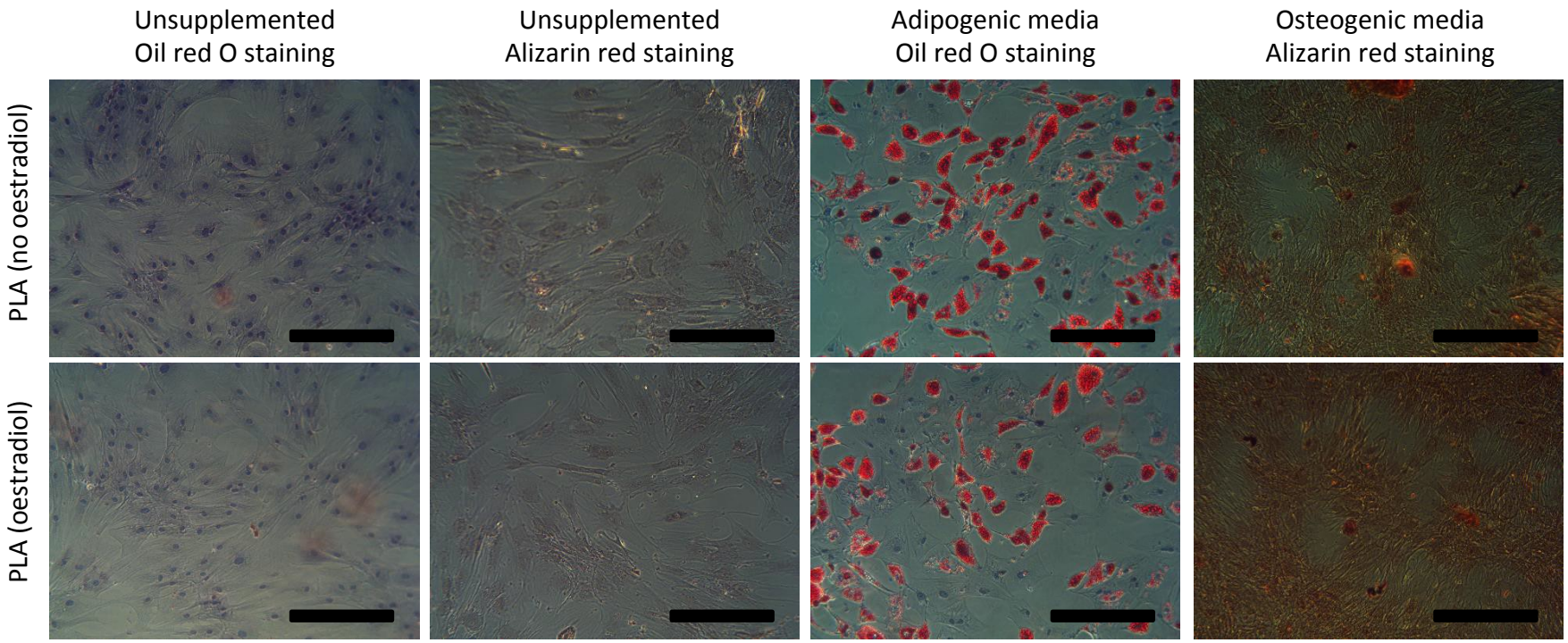


Figure 4.3.1. ***Cell differentiation assays in response to oestradiol***

ADSC cultured in either DMEM, adipogenic media or osteogenic media. Stained with haematoxylin and Oil red O (for lipidic vesicles) or Alizarin red (for Calcium). Scale

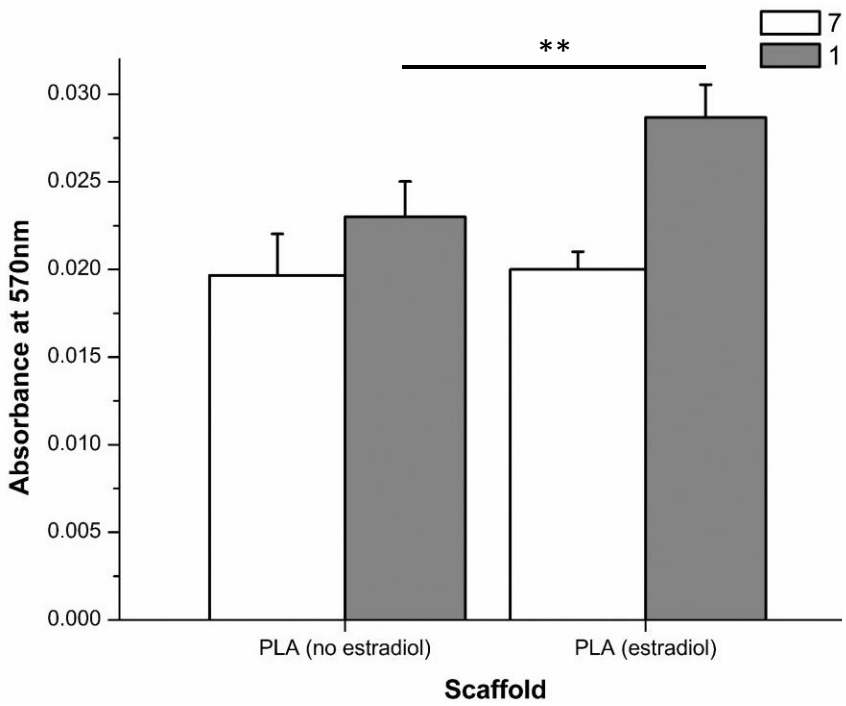


Figure 4.3.2. ***Cell metabolic activity of ADSC***

*Cultured on control and oestradiol releasing PLA scaffolds as measured by AlamarBlue assay with absorbance measured at 570nm using plate reading colourimeter. (n=6 ±SEM). **p<0.01*

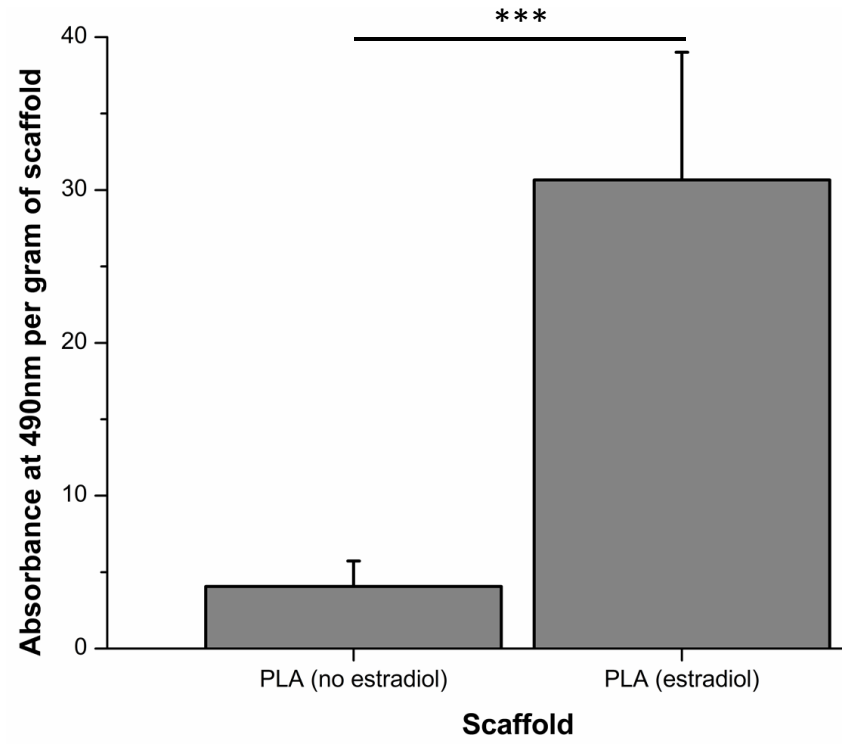


Figure 4.3.3. ***Total collagen production at day 14***

*As measured by Sirius red assay with absorbance measured at 490nm using plate reading colorimeter (n=6 ±SEM). ***p<0.001.*

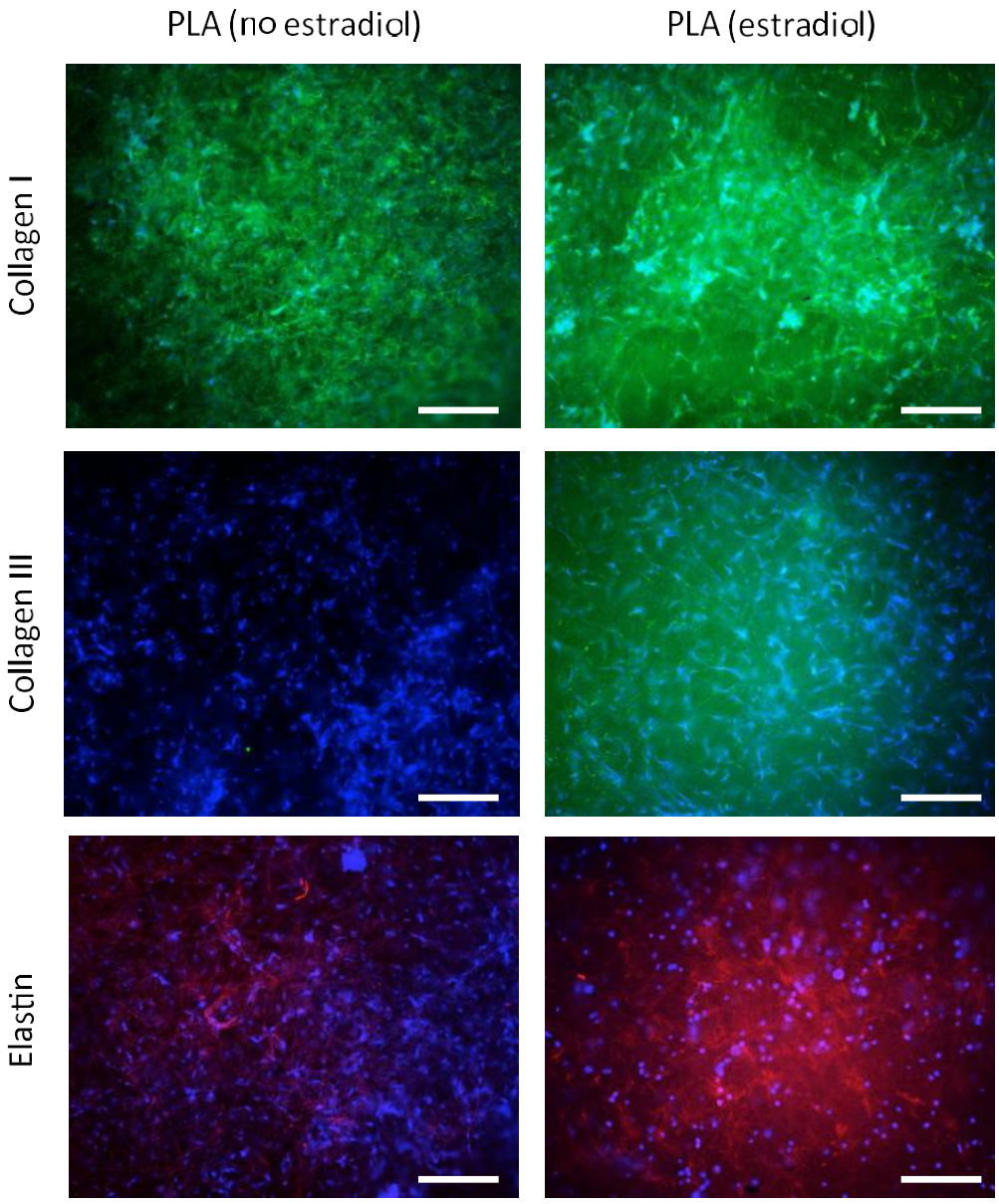


Figure 4.3.4. ***Representative fluorescent microscopy images of extracellular matrix components produced by ADSC after 14 days of culture***

Collagen I (green), elastin (red) and cells (blue). Scale bar represents 100µm.

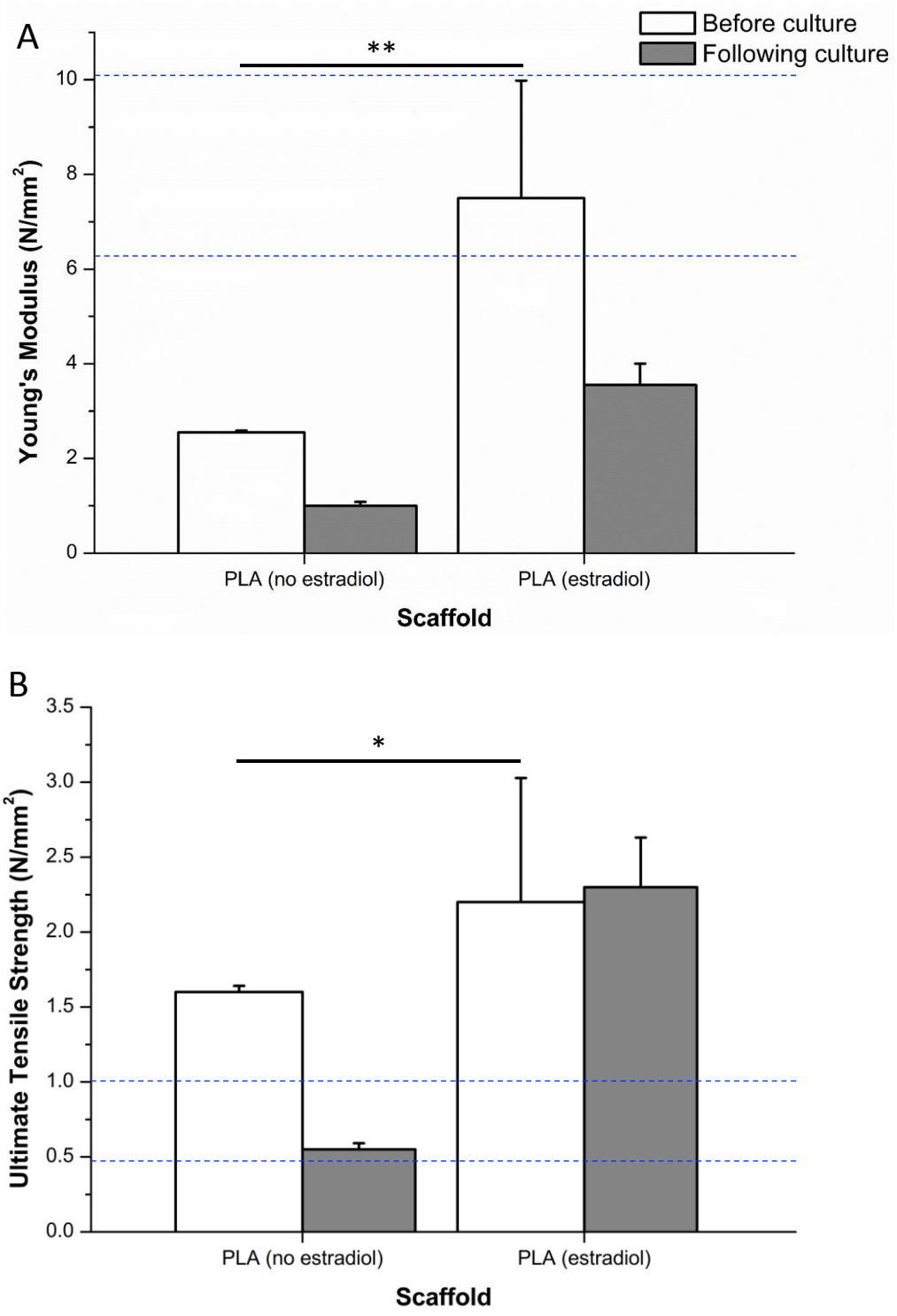


Figure 4.3.5. ***The effect of oestradiol of the strength of the scaffolds assessed in the presence of ADSCs***

*A) Young's Modulus of control and oestradiol releasing PLA scaffolds, before and after 14 days of cell culture with ADSC. Area between dashed lines represents values for healthy native fascia. n=6 ±SEM, **p<0.01. B) Ultimate Tensile Strength of control and oestradiol releasing PLA scaffolds, before and after 14 days of cell culture with ADSC. Area between dashed lines represents values for healthy native fascia. n=6±SEM, ***p<0.001.*

4.3.6 Section discussion

We demonstrate that cells cultured on scaffolds that release oestradiol show a significantly higher metabolic activity and production of total collagen than those scaffolds that do not release oestradiol. There does not appear to be any apparent toxic effects resulting from the oestradiol released from the scaffold over a two-week period. Furthermore, oestradiol does not stimulate cells to differentiate into an adipose or bone morphology. The most striking finding is that oestradiol is associated with a significant increase in the expression of collagen I, collagen III and also elastin. This increase in collagen could possibly explain the increase in tensile strength that is seen following 2 weeks of cell culture, while also becoming more elastic. Despite this, the presence of oestradiol in the scaffold fibres does lead to significant changes in the overall mechanical properties of the materials. However, when compared with healthy fascia, oestradiol-releasing PLA scaffolds are slightly stronger, yet more elastic.

4.4 Chapter discussion

The aim of this study was to develop an oestradiol releasing electrospun PLA scaffold, which was selected as an appropriate repair material to support deficient pelvic floor tissues. Oestradiol has been shown to become incorporated into PLA scaffolds to be released over a five-month period. The timing of the oestradiol release corresponds with fibre degradation, as PLA degrades over a 12-month period. This would indicate that the oestradiol has been incorporated into the polymer fibres of the scaffold itself, otherwise, an initial burst release of the drug would be observed, followed by minimal release thereafter. The initial burst release of oestradiol is purported to occur due to the presence of a drug outside the fibres of polymer when a blend method of drug delivery is used as in this case. This can be of concern when certain drugs are used that can create problems with toxicity in the short-term if used at high concentrations.

The initial burst release of drug seen in various studies owing to rapid diffusion of the investigated system can be overcome by alternative methods of electrospinning technique. Core-shell electrospinning, whereby two separate polymers are electrospun through the same co-axial nozzle produces a central core of drug-loaded polymer, inside an outer shell. This outer shell can be comprised of slowly degrading hydrophobic polymers that would otherwise permit the simple diffusion of drug observed in an initial burst release [388]. Other attempts at the control of drug release from electrospun scaffolds involves covalent coupling of drugs to the polymer prior to the electrospinning process or by coating of the drug releasing polymer fibres following this process [389, 390].

The time period over which the drug is released would coincide with the short-term healing phase of a wound bed following implantation of a material that releases a drug such as oestradiol. The concentration of drug that is released has been demonstrated over a two-week period to have no significant attenuation of the cell metabolic activity and this dose corresponds to the physiological concentration in pre-menopausal females. By altering the polymer, it is postulated that the release of the experimental drug could be made more rapid by using glycolic

acid [391] or more sustained by altering the material's wettability [392].

This *in vitro* work demonstrates that oestradiol has no adverse effects on these cells with respect to their metabolic activity, morphology or ability to differentiate into a range of phenotypes when subjected to their appropriate media. Furthermore, ADSCs when grown on oestradiol releasing PLA scaffolds produced significantly more extracellular matrix including collagen I, III and elastin than non-oestradiol releasing PLA scaffolds. The introduction of oestradiol did slightly reduce the mechanical properties of the PLA scaffolds but the Young's Modulus and UTS remain close to the values of healthy native fascia.

A limitation of this work is that a significant proportion of oestradiol is lost during the electrospinning process, which is therefore a relatively inefficient process to perform. Furthermore, the exact concentration of oestradiol that is present in the final scaffold material is difficult to exactly determine and is calculated based upon assumptions. Although oestradiol continues to be released from the PLA scaffolds at the end of the 5 month-release experiment, the rate at which it is released reduces significantly. Only by completely degrading the polymer scaffold and measuring the oestradiol concentration that is present would a more accurate measurement be feasible. However, PLA has been demonstrated to be present and intact after one year [393] and therefore conducting an experiment beyond this point is beyond the timescale of this project. Solvent degradation of the scaffold would accelerate this process, however the chemicals that are used during this process would interfere with the measurement of oestradiol.

Surgeons have historically used healthy native fascial slings for the treatment of SUI prior to the development of contemporary mesh surgery using PPL, which was subsequently repurposed for the treatment of prolapse. PPL mesh is associated with chronic inflammation and exposure in a small proportion of patients, a complication that is not associated with fascia and this is the basis for using healthy fascia as a reference material.

In conclusion, oestradiol when released from electrospun PLA can effectively

stimulate extracellular matrix production while keeping desired mechanical properties which predicts a better tissue integration *in vivo*.

Chapter V: in vivo assessment of repair materials

5.1 Chapter introduction

Thus far, we have assessed several degradable and non-degradable electrospun scaffold materials *in vivo*. The morphology, mechanical and cellular outcomes have been assessed, however how a material behaves following implantation requires the use of appropriate animal models. The most appropriate animal model to study repair materials for the treatment of pelvic floor conditions would be non-human primates, however this is clearly not a feasible option as an initial *in vivo* study. The main aims for these experiments are:

- To identify any macroscopic complications that occur following implantation.
- To assess the mechanical outcomes of implanted materials following implantation.
- To investigate the histological properties of the repair material following implantation.

The key objective with any short term *in vivo* implantation model is to assess the integration of the repair material into the host. This includes an assessment of the inflammatory response to the material, whether cells penetrate the material and whether these cells appropriately stimulate neomatrix production and angiogenesis. In small animals, it is likely that a 3-4 month implantation study would be sufficient to enable the assessment of these factors [363].

Based on the outcome data from the *in vitro* work, PLA and polyurethane Z3 were selected for implantation in this animal model. Several animal models have been used in this context, including rats [363, 394], sheep [264] and rabbits [294]. Based on the outcomes of previous rabbit experiments, if adverse outcomes were to develop, these would be evident by 60-90 days. Therefore an abdominal wall defect rabbit model was selected, with an implantation phase of up to 90 days. We purposely chose the rabbit model because: (1) rabbits allow a long follow-up period; (2) size-wise simultaneous implantation of several meshes within the same host is possible; (3) this species may challenge the long-term stability of collagen matrices, as rabbits are known to have a high collagenolytic activity [395].

The mechanical properties of the explanted material are perhaps not as important as

the mechanical properties of the *en bloc* repair site itself, including material and surrounding tissue. This is particularly true of tissues that are repaired using degradable materials that would be expected to become weaker over time, the strength of the repair site being provided by the tissue remodeling that occurs following implantation. Therefore, an assessment of both the explanted material and surrounding tissues is performed.

An important factor in the integration of a material is the macrophage phenotype present. An M2 macrophage response is indicative of constructive tissue remodeling [291] and is associated with cell infiltration, neovascularization and extracellular matrix production. These components are also tested in this study.

5.1.1 Material explantation

All rabbits survived the implantation stage of the study and there were no cases of overt infection over the course of the experiment. 8 rabbits per experimental group were sacrificed after 30 and 90 days, with the repair material and surrounding tissue explanted. PU scaffolds, PVDF and PPL meshes were easily identified on the abdominal wall with neo-tissue covering all materials (figure 5.1.1). PLA scaffolds however, were highly remodeled and became well-integrated into the host, which made the accurate identification of this material itself difficult. These scaffolds were identified by the presence of a non-absorbable suture, which was located at each of the four corners of the material. The main procedural complications were a small proportion of adhesions between the omentum and suture material (figure 5.1.2 and table 5.1), while mesh exposure was observed in two PVDF materials and 3 PPL meshes by 30 days (figure 5.1.3).

It is more likely that adhesions formed because of the peritoneal defect and the subsequent repair rather than the use of repair material, which is reflected in the incidence of peritoneal adhesions spread amongst the different groups of repair material.

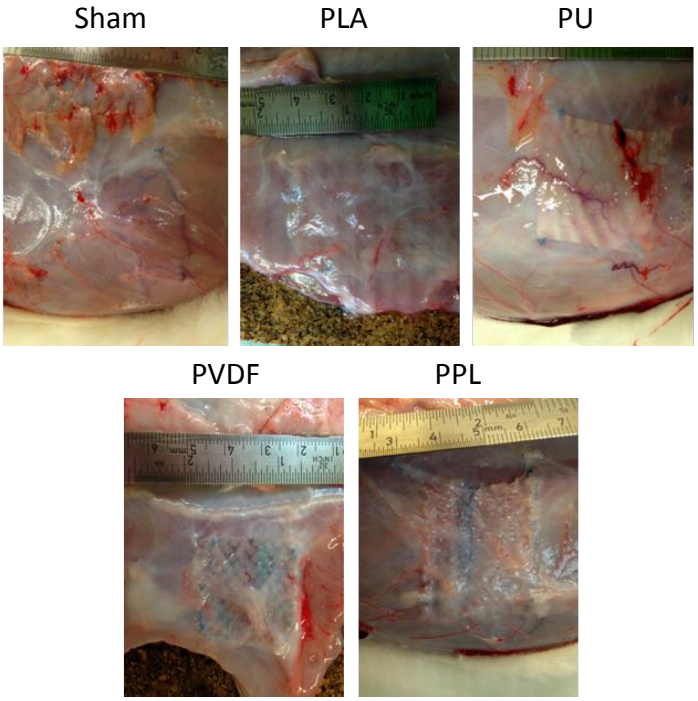


Figure 5.1.1. ***Macroscopic appearance of the materials after 90 days implantation***

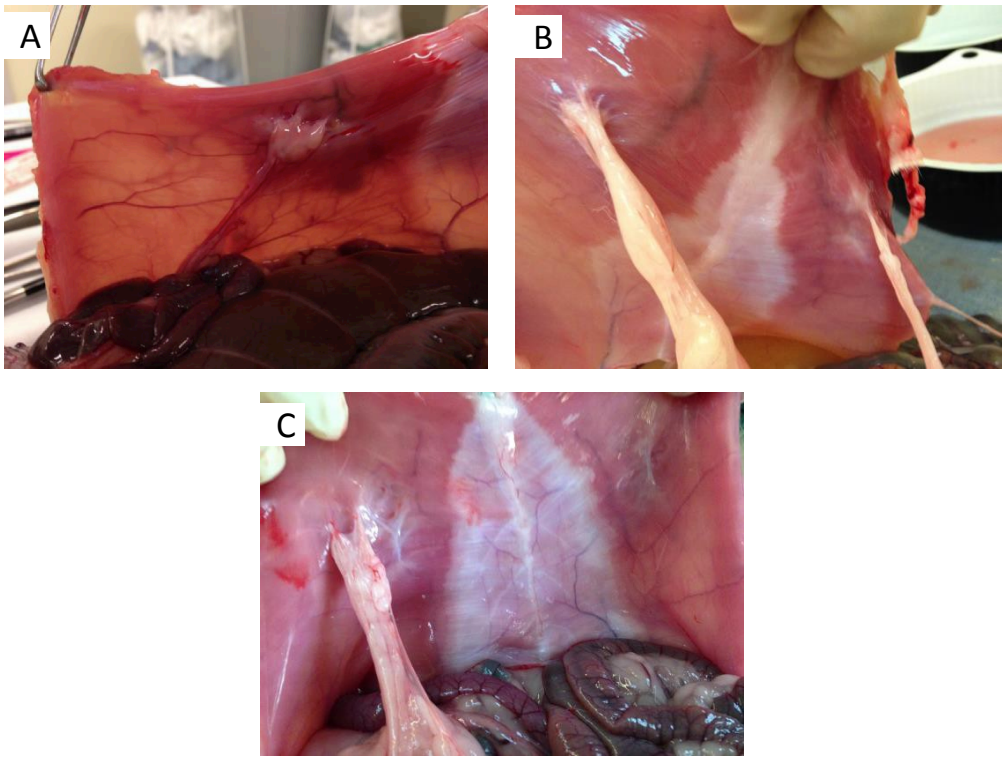


Figure 5.1.2. ***Adhesion formation between repair material and intra-abdominal organs at 30 days***

A) Sham repair. B) Polyurethane. C) Polypropylene.

Table 5.1. *Number of peritoneal adhesions occurring of 8 implants per group and time point*

Material	Day 30 (number)	Day 90 (number)
Sham	2	0
PLA	0	5
PU	3	0
PVDF	0	0
PPL	1	1
Total	6	6

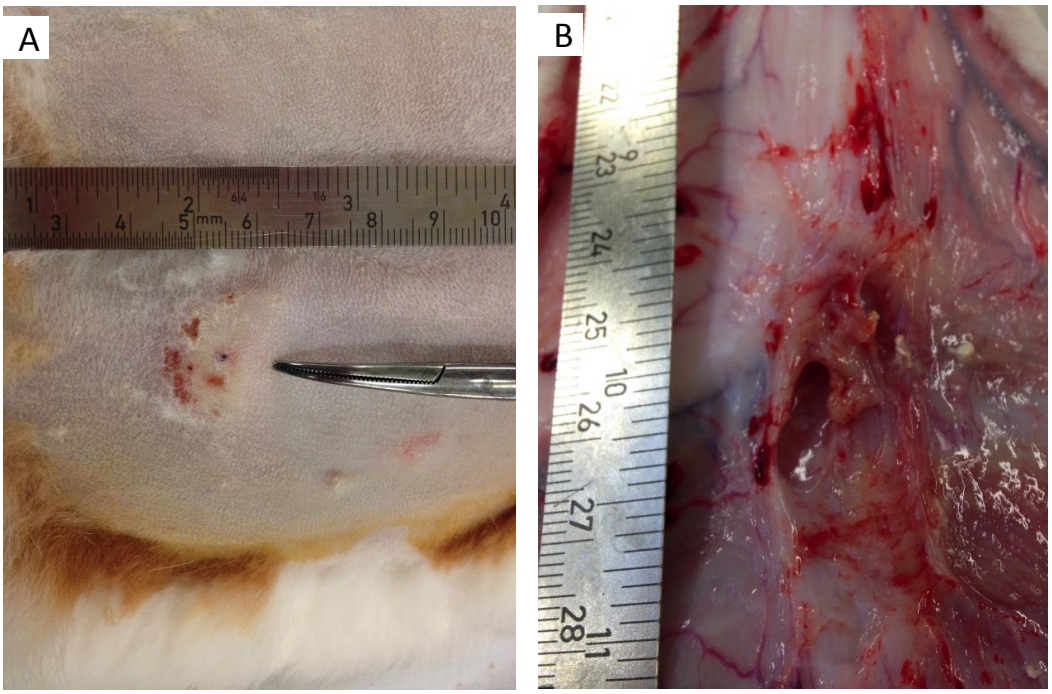


Figure 5.1.3. ***Polypropylene mesh exposure through rabbit abdominal wall***

A) External view. B) Internal view.

5.2 Mechanical outcomes

Mechanical properties of the dissected materials at 30 and 90 days were compared to the mechanical properties of the dry pre-implantation materials, which had a thickness of 300µm for PU and 100µm for PLA. These scaffolds were therefore three times thicker than those used during *in* vitro testing. The mechanical properties of the abdominal wall tissue that incorporated the implanted material at 30 and 90 days were compared to control tissue taken from non-operated healthy rabbit fascia and also to the sham repair sites. A ramp uniaxial test was performed for all samples, with the Young's modulus and ultimate tensile strength normalized to material thickness.

5.2.1 Mechanical properties of repair materials

To assess how the mechanical properties of the individual material behave following implantation, scaffolds and meshes were carefully dissected free of surrounding tissue. The location of the defect was identified during this process and care was taken to ensure that the portion of the repair material that was overlying the defect was tested. Individual statistical differences are demonstrated in appendix1.

5.2.1.1 Young's modulus

The Young's modulus of dissected repair materials is demonstrated in figure 5.2.1A. This shows that between 30 and 90 days, there was no significant change in Young's modulus for any of the tested materials (appendix 1). Both PLA and PPL however did demonstrate significant reductions in the Young's modulus after implantation, while the values for PU and PVDF remained unchanged.

5.2.1.2 Ultimate tensile strength

The ultimate tensile strength of dissected repair materials is demonstrated in figure 5.2.1B. This demonstrates that all materials show a reduction in UTS following implantation, however only PLA and PPL show a significant reduction in strength (appendix 1). Between 30 and 90 days, the UTS increased for all materials, a finding that did not reach statistical significance.

5.2.2 Mechanical properties of repair site

Biomechanical properties of the abdominal wall tissue incorporating the implant materials were assessed and compared to those of the abdominal wall in the sham group and in healthy controls. Mechanical testing of these samples was performed with the defect located at a central position. Controls include sham repairs (defect that was repaired with sutures but without any material) and healthy abdominal wall tissue. Individual statistical differences are demonstrated in appendix 2.

5.2.2.1 Youngs modulus

The Young's modulus of the repair sites for each of the tested materials at 30 and 90 days is demonstrated in figure 5.2.2A. The use of all materials resulted in a repair site with a higher Young's modulus than sham operated controls. While this finding did not reach statistical significance, the Young's modulus of healthy controls was significantly higher than any of the operated tissue at both 30 and 90 days. There were no significant differences between the experimental groups at 30 and 90 days as demonstrated by the Tukey multiple comparisons test (appendix 2).

5.2.2.2 Ultimate tensile strength

The ultimate tensile strength of the repair sites for each of the tested materials at 30 and 90 days is demonstrated in figure 5.2.2B. The UTS of all tested samples increased between 30 and 90 days, without any significant difference between any of the four experimental groups. Healthy controls had a significantly higher UTS than any of the experimental groups at both 30 and 90 days. The Tukey multiple comparisons test (appendix 2) demonstrated that amongst the four implant groups, the only significant finding was that PPL showed a higher UTS than the sham group at day 90.

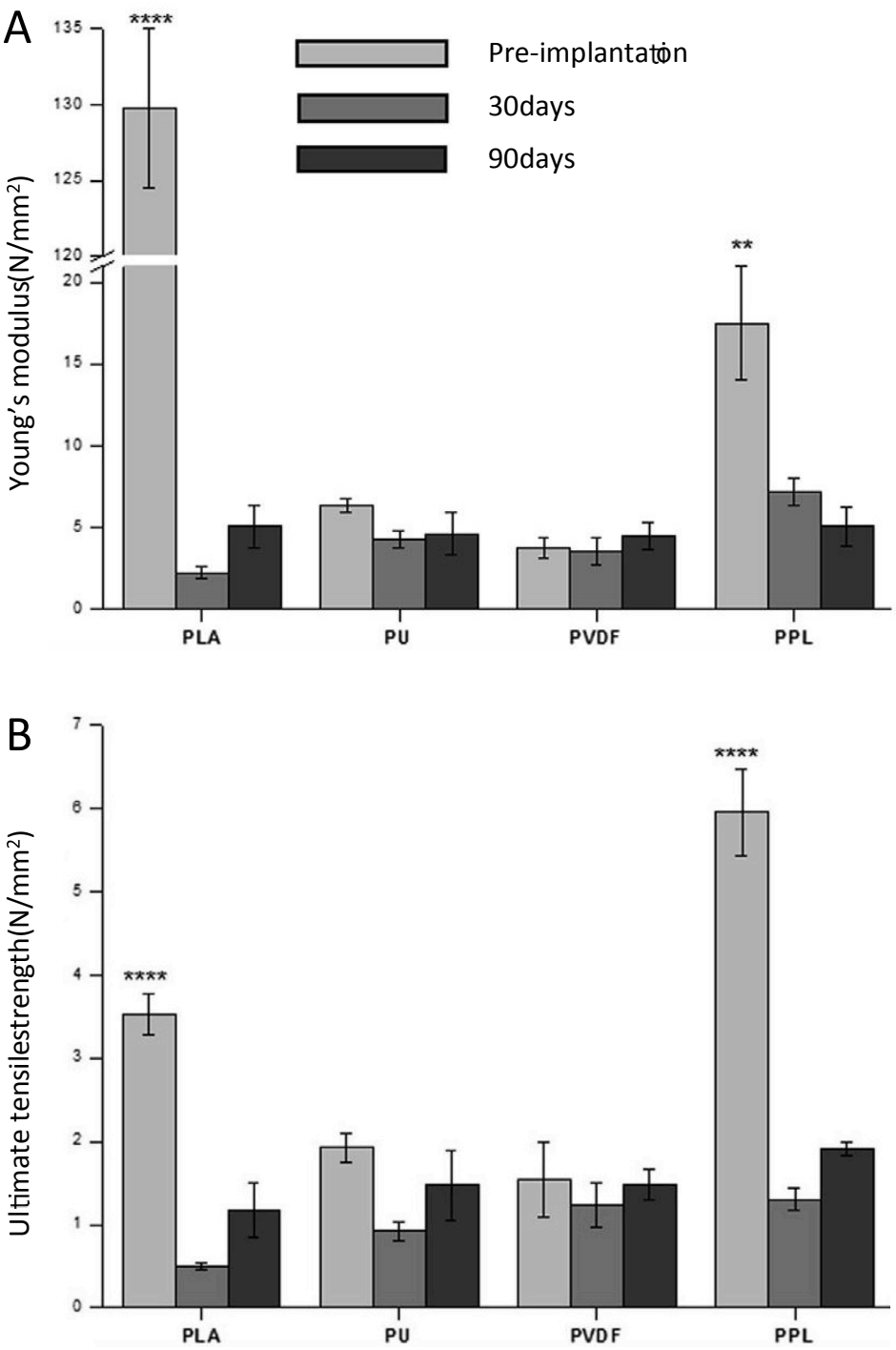


Figure 5.2.1. ***Mechanical properties of explanted repair materials***

*A) Young's modulus and B) ultimate tensile strength of 4 different materials at day 0, 30 and 90. N=4, mean ± SEM. ** p<0.01, **** p<0.0001.*

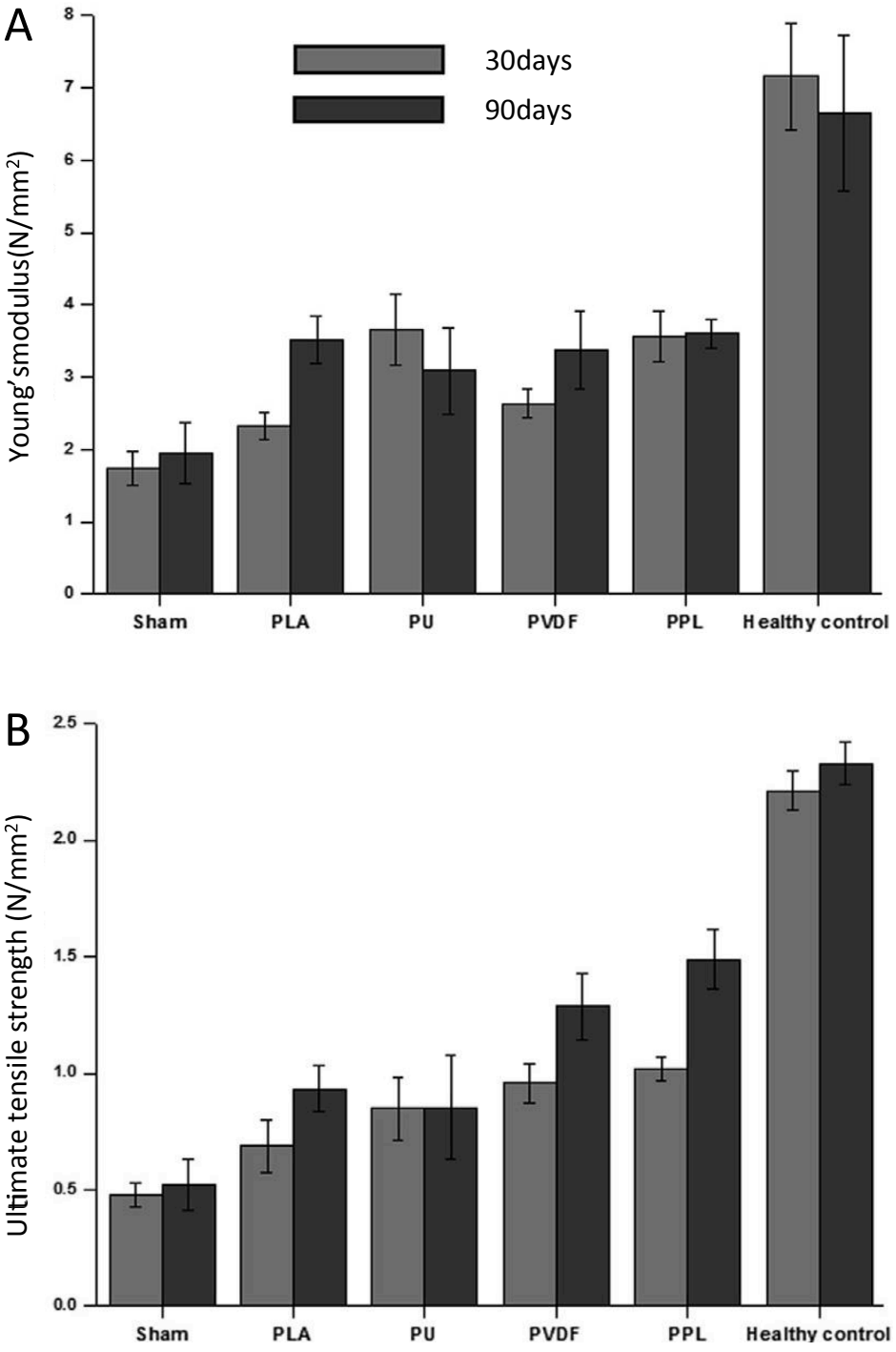


Figure 5.2.2. ***Biomechanical properties of explanted repair site***

A) Young's modulus and B) ultimate tensile strength of 4 different sites and two controls.

N=4, mean ± SEM.

5.2.3 Section discussion

The contemporary polypropylene mesh devices that are used for both SUI and POP surgery demonstrate plastic deformation and mechanical failure during routine cyclical distension [263, 382]. This finding is reported to be associated with the complications that are seen with the implantation of these devices [379, 396, 397]. We therefore investigate the mechanical properties of two alternative materials for the treatment of SUI and POP, a degradable (PLA) and non-degradable (polyurethane Z3) electrospun mesh. PLA demonstrates excellent cell integration and proliferation *in vitro*, while PU shows an absence of plastic deformation when subjected to repetitive distension. We investigate the mechanical outcomes of these investigational devices following implantation in an abdominal wall defect model over a 90 day period.

Following implantation, PLA becomes significantly more elastic but weaker, while the mechanical properties of PU remain unchanged. This is an important finding, which is likely to reflect the nature of the materials themselves; PLA undergoes degradation, while PU does not. Arguably a more accurate mechanical assessment is the testing of the site of repair, which has been augmented by the implantation of these investigational devices. While the use of these two materials result in a repair site that has a small but non-significant increase in UTS, the strength of the tissues is weaker than healthy controls. The interesting finding is that despite PLA undergoing degradation, the site of repair becomes stronger from 30 to 90 days. This is likely to reflect to ability of this scaffold to support cell proliferation and neomatrix formation, which replace the scaffold as it degrades over time.

Clearly, an abdominal wall defect rabbit model does not accurately mimic the human pelvic floor and it is therefore difficult to draw conclusions about how the mechanical properties of these materials will behave following their implantation in humans with SUI or POP. Despite this, it does not appear that any significant adverse outcomes exist following the implantation of these materials in abdominal wall defect male rabbit models. These investigational devices therefore require further pre-clinical testing and we currently await the findings of a 6-month assessment of PU Z3 that has been implanted into the vagina of sheep. It is difficult to seek central ethical

committee approvals to experiment on larger animals, which would be a much more accurate model of this particular application, furthermore, it would be significantly more expensive. Testing the host response to these implantable materials is an important initial step towards seeking further funding and approvals for testing in larger animals. Sheep are notoriously highly sensitive to the implantation of any biomaterial and therefore, this model was viewed to be a highly robust and sensitive measure of any site specific responses, such as inflammation or infection.

A further limitation of this experiment is that the mechanical outcomes are not tested at a time point that would correspond with further material degradation. We therefore cannot make conclusions on the strength of the repair site once PLA has completely degraded. However, figure 5.2.2 shows that the UTS of the repair site treated with PLA actually increases between 30 and 90 days, which corresponds with histological evidence of significant degradation of the material itself, a finding that will be further discussed in the following section.

5.3 Immunohistological outcomes

Mesh related complications do not develop in most patients, but a significant minority (currently estimated at 13%) do show severe complications, which suggests the importance of immune reactions, such as acute inflammation and other host processes, including neovascularization [259]. Indeed, the initial animal experiments performed for the assessment of PPL [398] demonstrated a wide range of host reactions, with some animals exhibiting integration of the material, while others showed a severe foreign body reaction. PPL mesh fibres ultimately become surrounded by areas of granulomatous inflammation that is triggered by macrophage infiltration, which are recruited to destroy the foreign body. With PPL, the mesh fibres therefore remain chronically inflamed. PLA is biodegradable and will therefore overcome this problem of ongoing chronic inflammation, becoming replaced with collagen as the PLA fibres undergo hydrolysis. The macrophage phenotype is used to identify whether an implanted foreign body is associated with ongoing chronic inflammation or constructive remodeling processes with the formation of structural neotissue.

We therefore assess these outcomes following 30 and 90 days. Explanted samples underwent fixation and immunohistological analysis, with objective values calculated based on blind scoring systems. Separate representative images are demonstrated for 90 days only. Appendix 3 demonstrates all immunological outcomes at 30 days, while appendix 4 shows the immunological outcomes at 90 days. Appendix 5 demonstrates the mean blind scoring values for each immunological outcome, the significance of which is shown in appendix 6 and 7 for 30 and 90 days respectively.

5.3.1 Sample integration

Figure 5.3.1 demonstrates H&E stained samples at 30 and 90 days. After 30 days, all materials demonstrated some degree of cellular infiltration and blood vessel formation (indicated by arrows), which had increased by 90 days. PLA became well integrated after only 30 days and appeared to be replaced with neotissue. PU showed cell infiltration from the deep surface of the scaffold at 30 days, which demonstrated progression by 90 days. PVDF and PPL fibres were surrounded by cellular infiltrates, however these fibres were also surrounded by a large proportion of loose connective

tissue. Sham operated controls demonstrated tissue remodeling, with appearances similar to healthy tissue by 90 days.

5.3.2 Collagen III production and macrophage infiltration

Figure 5.3.2 shows samples stained for collagen III at 90 days. This demonstrates that all samples show collagen III expression. Sham operated controls demonstrate a greater proportion of collagen III expression at the repair site, while for PPL and PVDF, there are small areas of darkly stained collagen around individual fibres surrounded by a much larger area of collagenous connective tissue. Meanwhile, the PLA scaffold had become completely replaced by collagenous tissue that has taken the appearance of bundles, similar to muscle. Individual PLA fibres are scarcely visible, while the PU scaffold is still identifiable, with high collagen III expression on the top and bottom surface of the scaffold.

Brown stained macrophages are present at the site of repair for sham operated controls (figure 5.3.3), while the remainder of explanted tissues show abundant macrophage infiltrates associated solely with the repair material itself. There are no macrophages present within samples of healthy tissue.

5.3.3 New blood vessel formation and lymphocyte infiltration

Figure 5.3.4 shows brown-stained endothelial cells throughout the explanted tissues at 90 days. For PU scaffolds, a much greater proportion of stained endothelial cells exists on the lower surface of the material, while PLA scaffolds have been replaced by tissue with endothelial cells present within it.

T-lymphocytes are not present within the two control groups, but are found scattered throughout all material repaired tissues (figure 5.3.5). Lymphocytes are found predominantly present within the regenerated PLA scaffold, while for PU, PVDF and PPL, lymphocytes are located surrounding the fibres only.

5.3.4 Macrophage phenotype

Figure 5.3.6 demonstrates the M1 (staining for HLA-DR) and M2 (staining for cd206) macrophage phenotype for all explanted samples at 90 days. There is an absence of

HLA-DR stain for control samples and small area of staining only for PLA and PU samples. PVDF and PPL however, demonstrate a much greater proportion of HLA-DR staining surrounding individual fibres, as compared to CD206 staining. PLA and PU meanwhile demonstrate a much greater proportion of CD206 staining, with small areas of sham operated controls stained brown.

Figure 5.3.7 demonstrates the blind scoring values for the M2/M1 macrophage ratio taken from several representative immunohistochemistry images similar to figure 5.3.6. This indicates that there is a much greater M2 response for healthy, sham, PLA and PU samples, with an equivocal result for PVDF and a predominantly M1 profile for PPL at 90 days. Based on these data, table 5.3 shows that PLA has a significantly greater M2 profile than any other tested material ($p<0.05$).

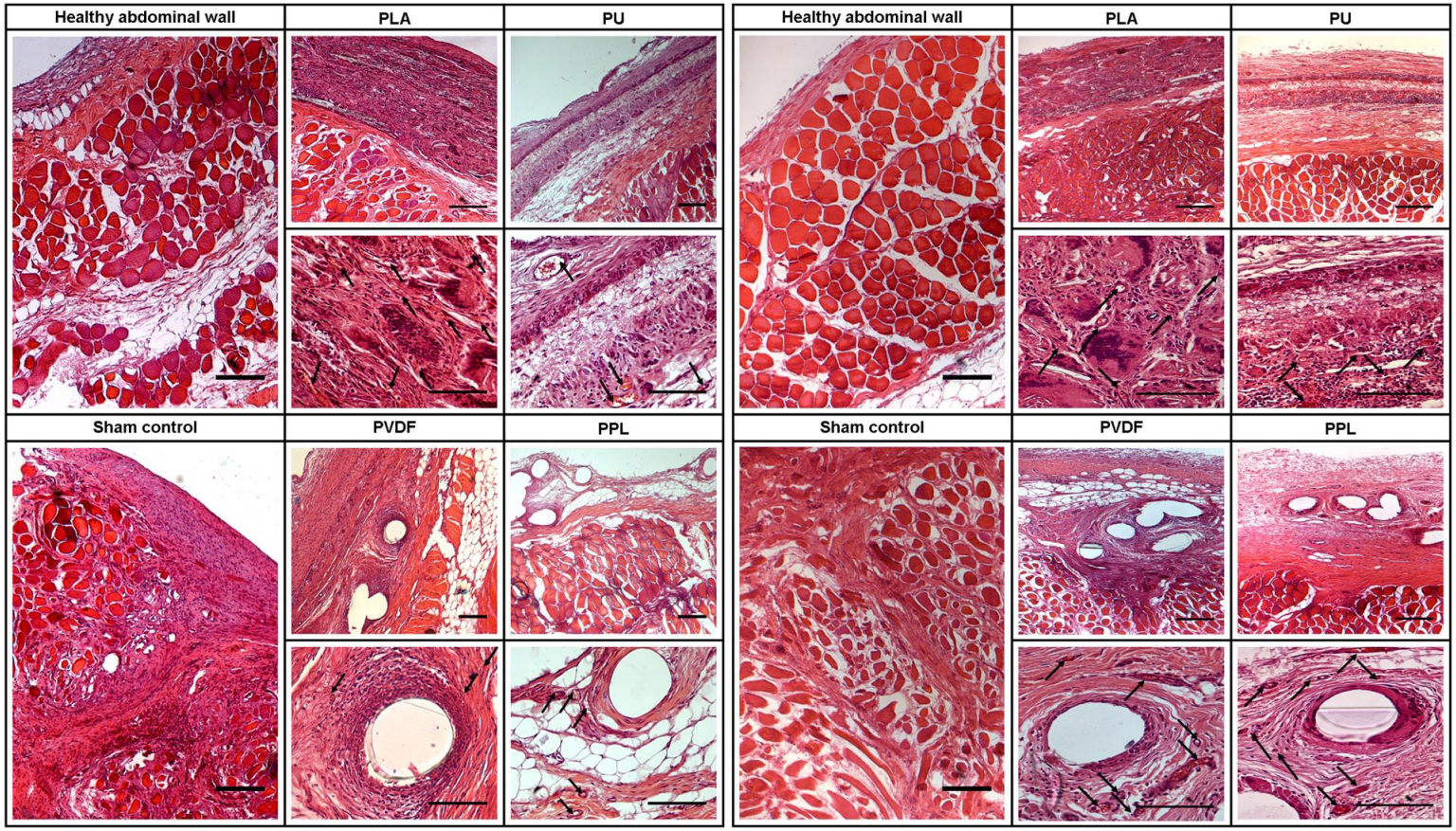


Figure 5.3.1. ***Histology of explanted samples***

(Left) at 30 days. (Right) at 90 days. Haematoxyllin and eosin staining. Scale bars indicate 0.1mm and 0.2mm for low and high magnificantion respectively

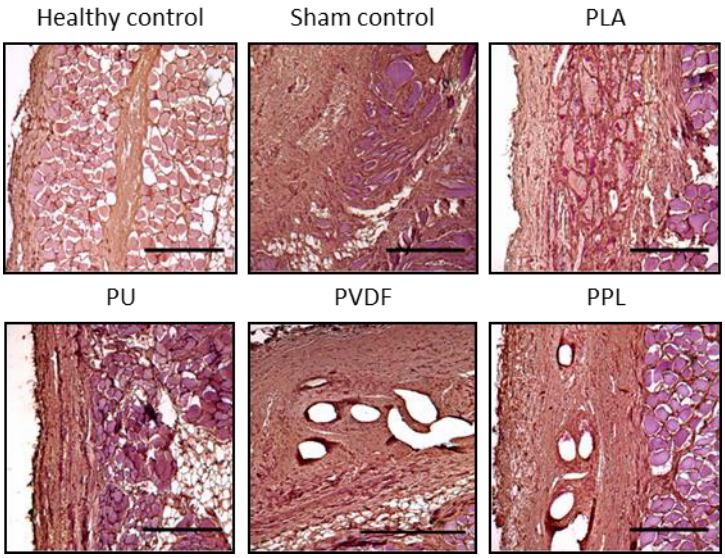


Figure 5.3.2. ***Immunohistochemistry staining for collagen III at day 90***

Collagen stained brown. Representative images from 4 samples. Scale bars represent 0.2mm

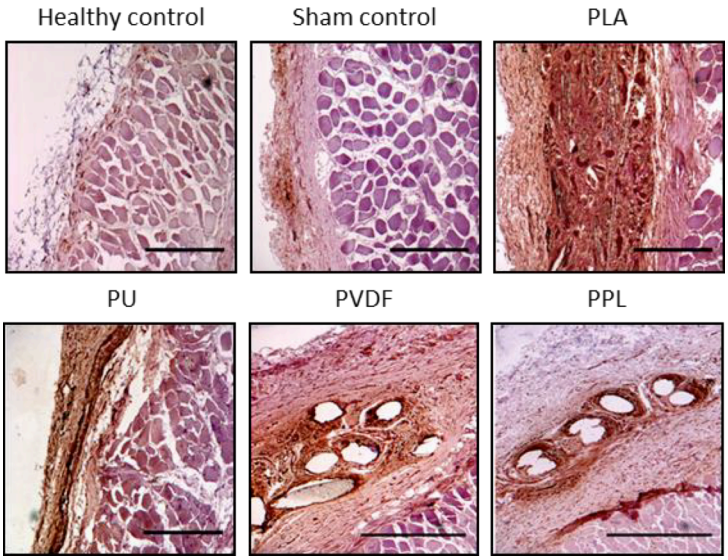


Figure 5.3.3. ***Immunohistochemistry staining for macrophages at day 90***

Macrophages stained brown using RAM 11. Representative images from 4 samples. Scale bars represent 0.2mm.

s

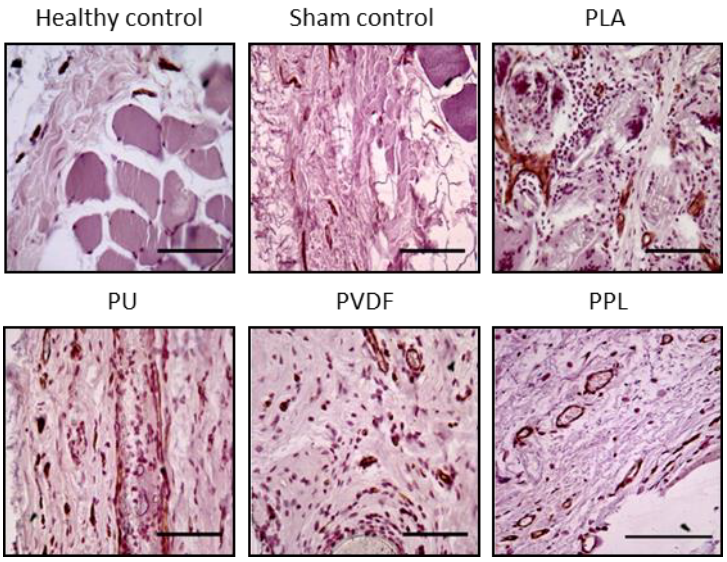


Figure 5.3.4. ***Immunohistochemistry staining for endothelial cells at day 90***

Endothelial cells stained brown using cd31. Representative images from 4 samples. Scale bars represent 0.1mm.

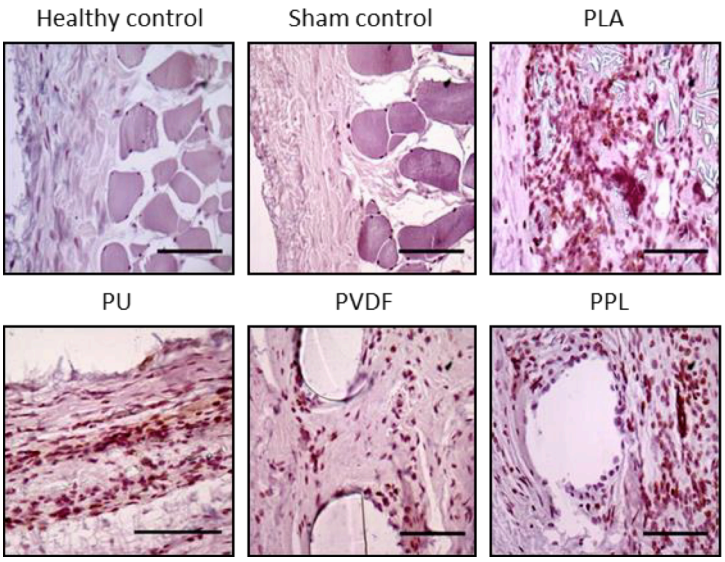


Figure 5.3.5. ***Immunohistochemistry staining for T lymphocytes at day 90***

T lymphocytes stained brown. Representative images from 4 samples. Scale bars represent 0.1mm.

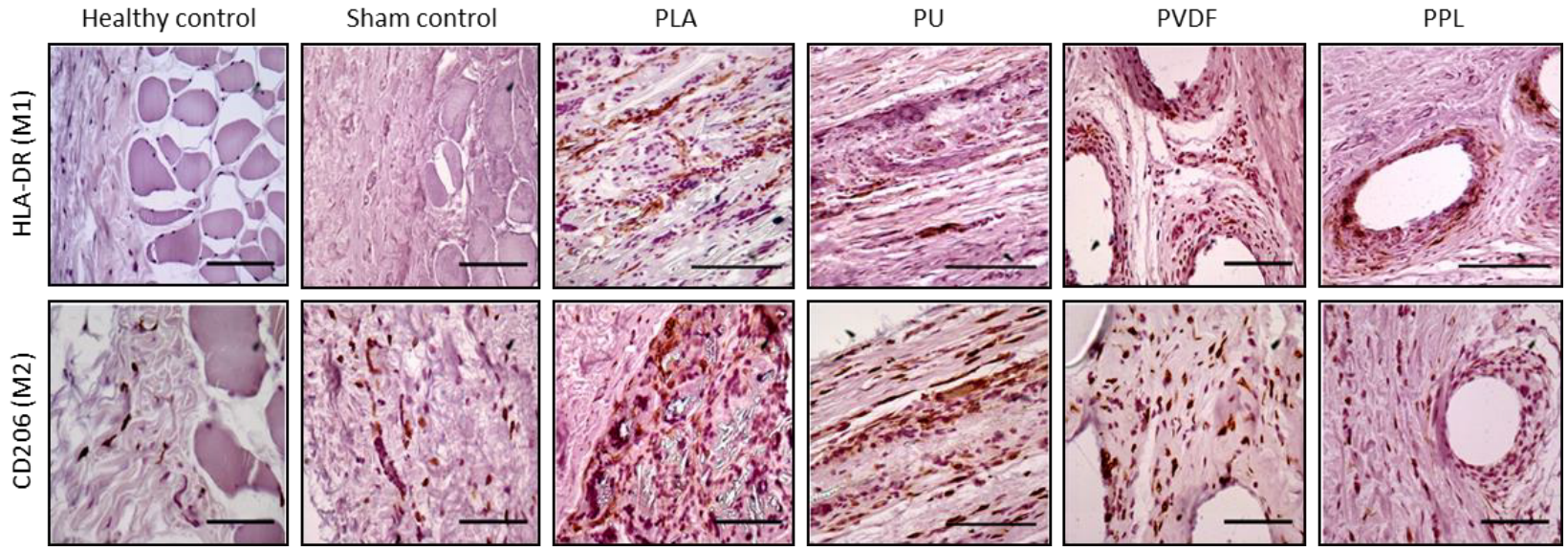


Figure 5.3.6. ***Immunohistochemistry images of M1 and M2 macrophage phenotype at day 90***

M1 (HLA-DR) and M2 macrophages (cd206) stained brown. Representative images from 4 samples. Scale bars represent 0.2mm.

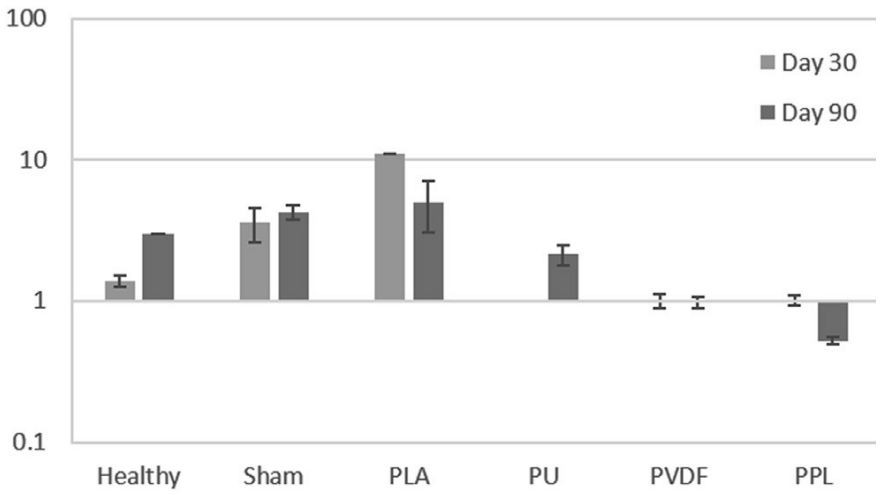


Figure 5.3.7. ***M2/M1 ratio for explanted materials***

12 representative images for each experimental group assessed by 6 blinded reviewers. Mean

Table 5.3. *Dunn multiple comparisons test of M2/M1 ratio taken from figure 5.3.7*

Experimental group		p value	
		30 days	90 days
Sham versus:	PLA, PU, PVDF, healthy control	ns	
	PPL	ns	<0.01
PLA versus:	PU, PVDF	<0.05	ns
	PPL	<0.05	
	Healthy control	ns	
PU versus:	PVDF, PPL, healthy control	ns	
PVDF versus:	PPL, healthy control	ns	
PPL versus:	Healthy control	ns	

ns. Not significant

5.3.5 Section discussion

Immediately following the implantation of a material, a biofilm rich in mitogens; cytokines and other growth factors surrounds the device, which activates an inflammatory response. This acute phase usually resolves within a week, followed by a chronic response that should not last more than a few weeks for biocompatible materials. Granulation tissue associated with neovascularization should mark the final process of the healing phase, with tissue remodeling as a result [256]. However, this is simply not observed in practice with the use of polypropylene.

The intention with degradable materials, such as PLA that has a favourable cellular response is that these processes can be overcome. With polyurethane, the mechanical properties are closer to those of the host tissue, and therefore, it is believed that the host response to that device will be much less pronounced than occurs with polypropylene.

Using this abdominal defect rabbit model, we demonstrate that by 90 days following implantation, both PLA and PU scaffolds become well integrated into the host as demonstrated by histology (figure 6.3.1). PLA is associated with a significantly greater M2 response that is indicative of constructive remodeling than any other tested material, while PPL and PVDF are associated with a significant M1 response that would suggest on-going chronic inflammation to these materials. Furthermore, in response to implanted PLA, host cells seem to express a significantly greater proportion of collagen III, an early indicator of tissue remodeling processes. While, polyurethane scaffolds are associated with constructive remodeling processes, albeit to a lesser degree than PLA, it does not seem that the material is associated with a chronic inflammatory response to the same extent as PPL or PVDF. We also demonstrate that macrophages are able to gain access to PLA, which is likely to occur as the material undergoes controlled degradation over the course of the experiment. All materials demonstrate evidence of new blood vessel formation, however only PLA shows that neovascularization that occurs within the area of the implanted material.

In summary, despite a much smaller pore size, both PLA and PU scaffolds

demonstrate host integration, with cell proliferation and matrix production *in vivo*, without any evidence of overt infection.

5.4 Chapter discussion

Other authors have described the use Human ADSC as a cell source for this study as these cells are more proliferative and better defined than fibroblasts and they are capable of contributing to wound healing in several ways making them a popular cell for tissue regeneration [325]. This particular rabbit model was used for the assessment of repair materials and report on the mechanical and cellular outcome data of these devices [294]. Although the stresses that occur in the rabbit abdomen clearly do not represent the pressure changes that occur within the female pelvic floor, this model provides an assessment of the mechanical behavior of tested materials in response to host processes and over a much longer period of time than can be feasibly demonstrated in a laboratory setting. Despite this, the exact pressure changes within the rabbit abdominal cavity are not known and it is likely that there will be some degree of stress at the repair site. What is clear, however is that full-thickness defects that are repaired with sutures alone are significantly weaker than those repaired by any of the tested materials, which in turn are weaker than healthy rabbit abdominal wall. It is entirely feasible that if the experiment were to be conducted over a longer time period then the tissue strength of repaired defects would increase further towards the levels seen for non-operated controls, as remodeling processes occur over a longer duration [399]. PLA demonstrates that this process occurs, showing an increase in UTS from 30 to 90 days despite histological evidence of degradation with a corresponding increase in collagen fibre expression throughout the remodeling material.

Both commercially available PPL and PVDF meshes were included as reference materials in these experiments, to reflect the current climate of the market. There are many variants of these two materials available that differ in their mesh patterns and orientation. Essentially we are comparing two new microporous mesh devices against two established non-degradable Amid type I mesh devices.

Areas of healthy abdominal wall were taken from all rabbits to ensure that the implantation of one material in the abdominal wall did not cause systemic effects, i.e evidence of systemic inflammation or tissue weakening. There was no evidence to

suggest that this occurred and the mechanical properties of healthy controls actually increased from 30 to 90 days.

Polyurethane Z3 is considered to be a non-biodegradable polymer. Despite this, the manufacturer claims that this material will actually degrade over a 10 year period, a process that may occur faster when the material is subjected to host mechanisms in the body. Clearly, as neither PLA nor PU scaffolds degraded over the course of the study, it is not possible to draw any conclusions on the relationship between material degradation and mechanical outcomes from these studies. Furthermore, there was insufficient funding to extend this study beyond the 90 day time frame. Further studies in sheep beyond 6 months are planned for the future.

Another limitation of this experiment is with the relatively short duration of implantation. It has been demonstrated that the complications, which occur with non-degradable mesh can take many years to occur [400] [253] and therefore a 3- month model may not show evidence of this phenomenon. However, of those patients who do develop complications after many years following implantation, it is unlikely that there is an initial period of resolution, rather there is the persistence of a chronic inflammatory process until such a time that it becomes clinically apparent.

Clearly, a more accurate model of SUI is required to formally assess repair materials in this context. Due to the animal's size, it is simply not feasible to perform anti-incontinence or prolapse surgery in rabbits. The female rabbit vagina is far too small to place a sample of sufficient size to allow adequate testing. Therefore, the abdominal wall was a selected as an appropriate site to asses host responses and the mechanical outcomes of materials following implantation.

Ultimately, with the implantation of materials that the body is unable to remodel, such as PPL mesh, although macrophages and other inflammatory cells are better able to penetrate the material, the macrophage phenotype is associated with a persistent foreign body response and excessive fibrosis [401]. Only by achieving a device that mimics the host tissues will this response to the implanted material be more desirable [402]. We therefore selected these two materials; PLA that provides a

suitable cellular environment and PU that closely resembles the mechanical properties of host tissues [382].

Chapter VI: Final discussion and conclusions

6.1 Discussion

Regenerative medicine and tissue engineering approaches to the treatment of pelvic floor disorders can offer a solution to many of the drawbacks of current repair materials. With current PPL meshes, infection, dyspareunia and pain can result and patients may ultimately need to undergo remedial surgery. Mesh exposure, seen in a small but significant proportion of patients can result from a persistent inflammatory and fibrotic reaction in response to implanted foreign materials and occurs to a greater extent with POP surgery, where a much larger surface area of mesh is required [1]. Furthermore, it seems that this stiff and inelastic material may not be as appropriate to the pelvic floor environment as it is for a hernia repair, where the material works well.

Autologous fascia, taken from the rectus or fasica lata of the thigh has been used for many years for the treatment of SUI. The benefit with autografts is that the excessive immune reactions that occur with the implantation of PPL do not develop. However, the harvest of autologous fascia adds additional time to the operation and can occasionally result in donor site morbidity. Mesh tape surgery was developed in the mid-1990s in an attempt to reduce recovery times.

Electrospinning is a relatively simple and cheap procedure to produce scaffolds for cell attachment, growth and neomatrix formation. While a variety of syringe pumps, mandrels and voltage devices can be purchased for this purpose, the cost of a basic electrospinning rig such as that used in these experiments is the equivalent to £2000. Various conditions can influence the characteristics of produced materials. By using a rotating collector, aligned polymer fibres can be formed that confer material strength in certain orientations, whereas randomly arranged fibres can allow greater cell penetration. Fibres of varying diameter can be achieved by altering the voltage applied or the viscosity of the polymer solution. This can subsequently have an effect on the fibre degradation rates.

Rapidly degrading polymers would seem ideal for use as drug delivery systems to cover the initial healing phase of a wound bed following implantation, however, the

material may degrade before an adequate regeneration of host tissues is complete, resulting in inadequate support. Slowly degrading polymers (PU or polycaprolactone) can offer an improved mechanical profile, however, their hydrophobicity prevents adequate cell attachment [403].

The evidence suggests that both fibroblasts and mesenchymal stem cells perform similarly when cultured on scaffolds in terms of attachment and proliferation. Vaginal fibroblasts would intuitively seem the most appropriate cell source, however research has demonstrated these cells to be deficient in structural components and enzymatic pathways in patients with POP [128]. To avoid the host response and potential disease transmission, autologous cells, particularly the relatively accessible ADSC would seem the most promising cell candidate.

Mechanical stimulation of produced scaffolds has been shown to lead to an increase in the extracellular matrix production and a variety of techniques have been investigated in order to demonstrate this [404]. We have demonstrated that the mechanical properties of polyurethanes remain unaltered following cycles of continuous mechanical stimulation, however, we have not yet examined whether proportions of the extracellular matrix components increase. Furthermore, as the demands exerted on scaffolds following implantation are not only in the uniaxial plane, testing of these materials solely in this fashion does not reflect the demands that will be placed on it once implanted in the body.

The role of electrospinning for delivery of drugs to treat SUI and POP is a relatively recent advancement with several potential therapeutic targets. Oestrogen, an obvious trophic substance for release into the pelvic floor environment has been investigated in several studies showing improvements in local ECM component production. There is evidence however that oestrogen can inhibit elastin expression and precipitate POP when administered parenterally [405]. Several substances would be of benefit for release from scaffolds into a variety of tissues, for example non-steroidal anti-inflammatory drugs, antibiotics, anti-oxidants or growth factors. Although simple methods of drug incorporation are well established such as drug-blending and co-axial electrospinning, the exact surface

chemistry and specific dose delivery of drugs is difficult to control. One solution is to induce functional groups in/onto scaffolds, while another is to photo-cross-link substance-scaffolds in order to provide more material stability and assist in the binding of substances, particularly growth factors.

Appropriate animal models to simulate POP are predominantly limited to non-human primates, which many European groups refrain from using due to ethical constraints. These subjects would more accurately reflect the forces experienced in women due to an upright posture and comparable organ/tissue dimensions. Alternatively, immune responses are typically studied in subcutaneously implanted mice, with host integration in abdominal wall reconstruction models using rats or rabbits, allowing passive biomechanical measurement.

Animal models that mimic POP and SUI over a short period of time would be imperative in order for tissue engineering techniques for the treatment of pelvic floor disorders to progress. The treatment of a benign and non-life threatening condition with potentially hazardous implants should be carefully studied before clinical trials can be considered in human subjects.

The learning curve associated with conducting and performing these experiments was roughly six months in total. The majority of the first year of this 3 year programme was spent developing new techniques and re-learning biological principles. I was fortunate to have the support of two post-doctoral researchers, who taught me the basic principles of cell culture, cell seeding, along with the routine tests involved in this research. The techniques of electrospinning were already well established in our department, however it did take several months for me to reproducibly produce consistent experimental materials.

Clearly, as with any cell based research failed experiments do occur. Several of the cell assessment methods take up to three weeks to achieve results and I rapidly realized that in order to avoid significant delays, I would perform all experiments at least in triplicate. Cell infection was the leading cause of such delays and this is often difficult to avoid during incubation, where cells are stored along with the work of

many other junior researchers and students. When this did occur, experiments were terminated. This often meant that a significant proportion of work required repeating; a problem that is not only demoralizing, but impacts upon the progress of other experiments and increases the cost of the project.

All of the methods performed in this research project were directly performed by me, with the exception of the device implantation in animals, which was conducted by colleagues in Belgium. The mechanical and histological assessment of the animal experiment outcomes took five months to perform and therefore this was performed with collaboration with a colleague. Several of the underlying principles of this work have subsequently been used in other student projects, such as the use of oestradiol to induce neovascularization and the use of polyurethane scaffolds as vascular grafts

Overall, I developed a wealth of novel scientific knowledge relating to stem cell biology, engineering techniques and assessment methods, not to mention the multitude of assessment techniques that I learned over the course of this 3 year project. This programme also allowed me to become a better researcher and develop other transferable techniques, such as statistical analysis methods, information technology skills and critical thinking.

6.2 Conclusions

Electrospun poly-L-lactic acid scaffolds are associated with a significantly better inflammatory profile than the currently used polypropylene mesh. Furthermore, following implantation, the remodeled tissues assimilate the load bearing properties of the degrading polymer fibres. The use of polyurethanes as scaffolds for the treatment of stress urinary incontinence and pelvic organ prolapse can offer improved biomechanical properties as compared to other potential synthetic materials. These new materials can be fabricated in order to tune their elasticity and strength, and be combined with other materials that provide a better material for cell attachment and proliferation. Certain biomimetic substances can be incorporated into these polymer fibres, which are then released corresponding with the material degradation. Oestradiol can lead to a significant increase in total collagen production of cells cultured on these scaffolds, yet does not lead to any toxic effects on the cells.

Testing of the immunogenic response to these implants is crucial. The polyurethane containing scaffolds are planned to be implanted the vagina of sheep to assess their mechanical properties following implantation along with the inflammatory response (macrophage response), cell integration and rate of material degradation.

6.3 Future directions

It is very likely that there will be a continuing fall in the number of patients who undergo vaginal mesh surgery. This may be associated with the withdrawal of several implants from the market in response to class action lawsuits, a growing public awareness of the complications that can occur, or a reluctance amongst surgeons to implant these devices. Therefore, the development of a new pelvic floor repair material could offer a much needed benefit to patients.

Prior to testing any new material in human subjects, it is imperative to undertake animal testing in more appropriate models, over a much longer time period. Therefore, we have recently begun to implant polyurethane Z_3 scaffolds into the vagina of sheep, over a 6-month period and we currently await the final results of the mechanical and cellular outcomes from this experiment. Furthermore, we aim to conduct an assessment on sterilization protocols and methods for packaging, with an investigation into the long-term stability of these devices. Once appropriate safety studies have been completed, an initial feasibility study in small numbers of patients with SUI or POP can be contemplated.

Appendices

Appendix 1

Tukey's multiple comparisons test for significance. Young's modulus of dissected materials.

YM Dry	Significance	YM 30 days	Significance	YM 90 days	Significance
PLA vs. PU	****	PLA vs. PU	ns	PLA vs. PU	ns
PLA vs. PVDF	****	PLA vs. PVDF	ns	PLA vs. PVDF	ns
PLA vs. PPL	****	PLA vs. PPL	ns	PLA vs. PPL	ns
PU vs. PVDF	ns	PU vs. PVDF	ns	PU vs. PVDF	ns
PU vs. PPL	*	PU vs. PPL	ns	PU vs. PPL	ns
PVDF vs. PPL	**	PVDF vs. PPL	ns	PVDF vs. PPL	ns
YM from dry to 30 days	**Significance**	**YM from dry to 90 days**	**Significance**	**YM from 30 to 90 days**	**Significance**
PLA	****	PLA	****	PLA	ns
PU	ns	PU	ns	PU	ns
PVDF	ns	PVDF	ns	PVDF	ns
PPL	ns	PPL	**	PPL	ns

Tukey's multiple comparisons test for significance. UTS of dissected materials.

UTS Dry	Significance	UTS 30 days	Significance	UTS 90 days	Significance
PLA vs. PU	*	PLA vs. PU	ns	PLA vs. PU	ns
PLA vs. PVDF	**	PLA vs. PVDF	ns	PLA vs. PVDF	ns
PLA vs. PPL	****	PLA vs. PPL	ns	PLA vs. PPL	ns
PU vs. PVDF	ns	PU vs. PVDF	ns	PU vs. PVDF	ns
PU vs. PPL	****	PU vs. PPL	ns	PU vs. PPL	ns
PVDF vs. PPL	****	PVDF vs. PPL	ns	PVDF vs. PPL	ns
UTS from dry to 30 days	**Significance**	**UTS from dry to 90 days**	**Significance**	**UTS from 30 to 90 days**	**Significance**
PLA	****	PLA	****	PLA	ns
PU	ns	PU	ns	PU	ns
PVDF	ns	PVDF	ns	PVDF	ns
PPL	****	PPL	****	PPL	ns

ns – not significant

* - p<0.05

** - p<0.01

*** - p<0.001

**** - p<0.0001

Appendix 2

Tukey's multiple comparisons test for significance. Young's modulus of entire abdominal wall repair.

YM 30 days	Sig.	YM 90 days	Sig.	YM from 30 to 90 days	Sig.
Sham vs. PLA	ns	Sham vs. PLA	ns	Sham	ns
Sham vs. PU	*	Sham vs. PU	ns	PLA	ns
Sham vs. PVDF	ns	Sham vs. PVDF	ns	PU	ns
Sham vs. PPL	ns	Sham vs. PPL	ns	PVDF	ns
Sham vs. Healthy	****	Sham vs. Healthy	****	PPL	ns
PLA vs. PU	ns	PLA vs. PU	ns	Healthy	ns
PLA vs. PVDF	ns	PLA vs. PVDF	ns		
PLA vs. PPL	ns	PLA vs. PPL	ns		
PLA vs. Healthy	****	PLA vs. Healthy	****		
PU vs. PVDF	ns	PU vs. PVDF	ns		
PU vs. PPL	ns	PU vs. PPL	ns		
PU vs. Healthy	****	PU vs. Healthy	****		

Tukey's multiple comparisons test for significance. UTS of entire abdominal wall repair.

UTS 30 days	Significance	UTS 90 days	Significance	UTS from 30 to 90 days	Significance
Sham vs. PLA	ns	Sham vs. PLA	ns	Sham	ns
Sham vs. PU	ns	Sham vs. PU	ns	PLA	ns
Sham vs. PVDF	ns	Sham vs. PVDF	ns	PU	ns
Sham vs. PPL	ns	Sham vs. PPL	*	PVDF	ns
Sham vs. Healthy	****	Sham vs. Healthy	****	PPL	ns
PLA vs. PU	ns	PLA vs. PU	ns	Healthy	ns
PLA vs. PVDF	ns	PLA vs. PVDF	ns		
PLA vs. PPL	ns	PLA vs. PPL	ns		
PLA vs. Healthy	****	PLA vs. Healthy	****		
PU vs. PVDF	ns	PU vs. PVDF	ns		
PU vs. PPL	ns	PU vs. PPL	ns		
PU vs. Healthy	****	PU vs. Healthy	****		
PVDF vs. PPL	ns	PVDF vs. PPL	ns		
PVDF vs. Healthy	***	PVDF vs. Healthy	**		
PPL vs. Healthy	***	PPL vs. Healthy	*		

ns – not significant

* - $p<0.05$

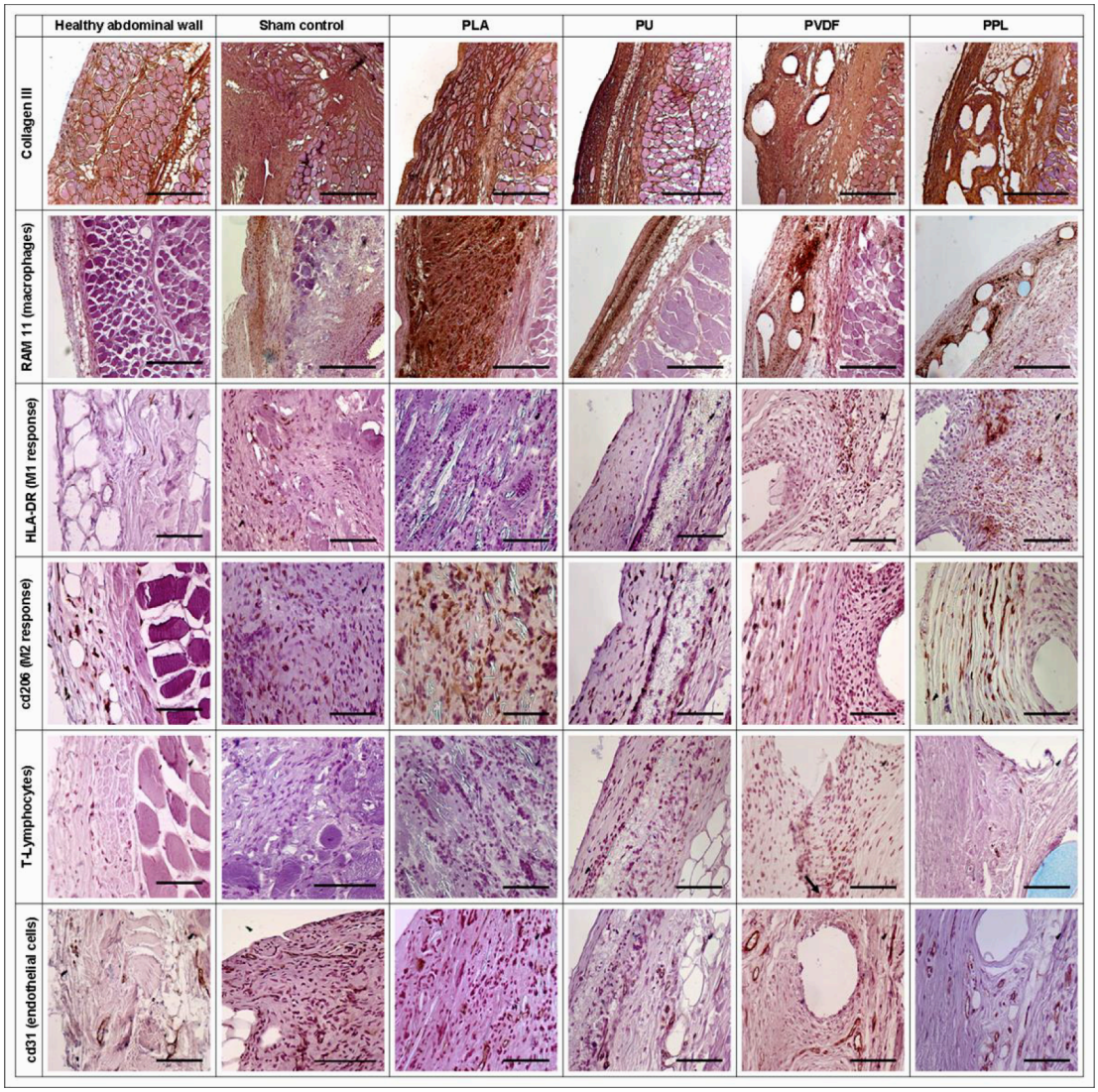
** - $p<0.01$

*** - $p<0.001$

**** - $p<0.0001$

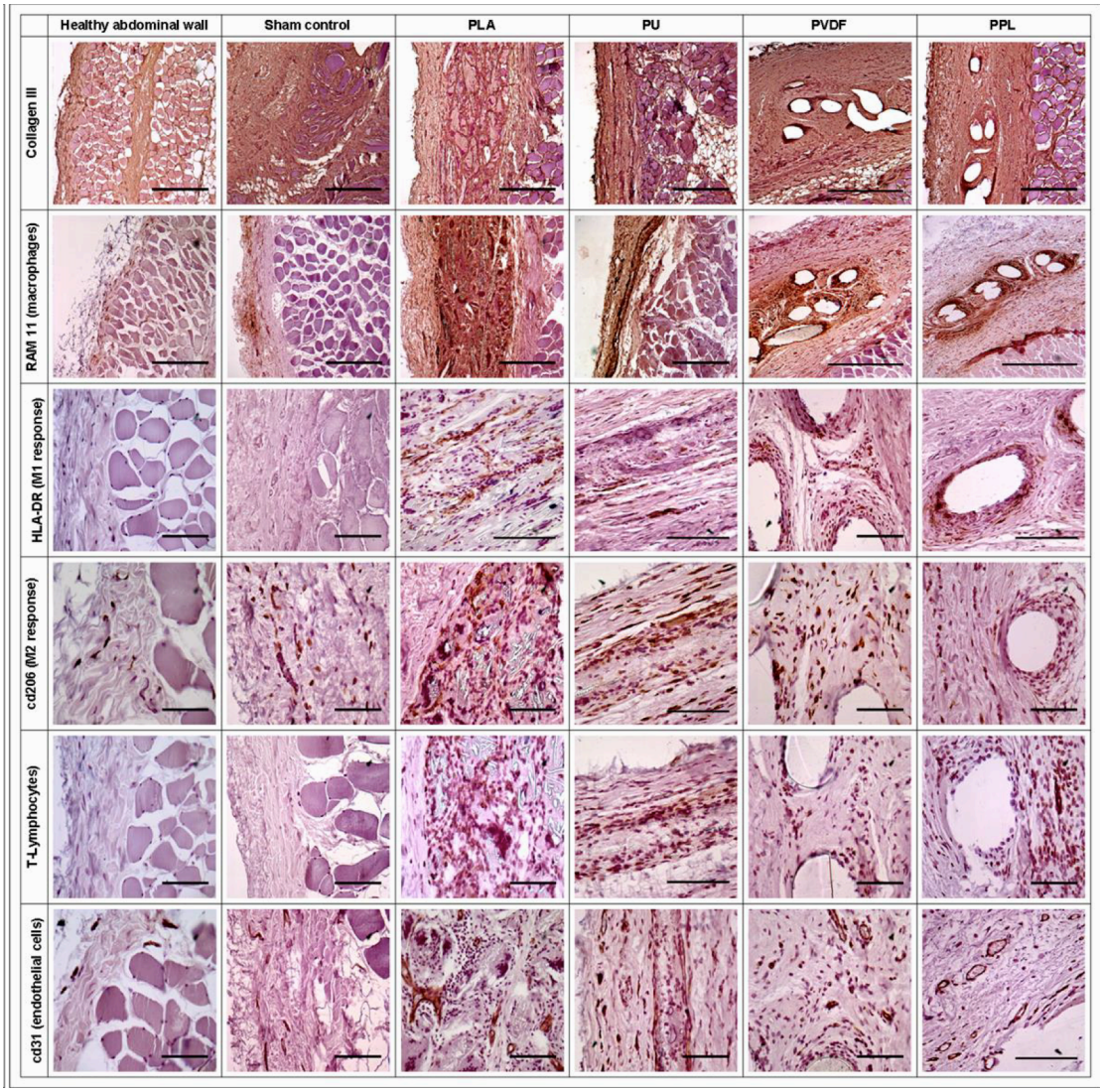
Appendix 3

Immunological outcomes at 30 days

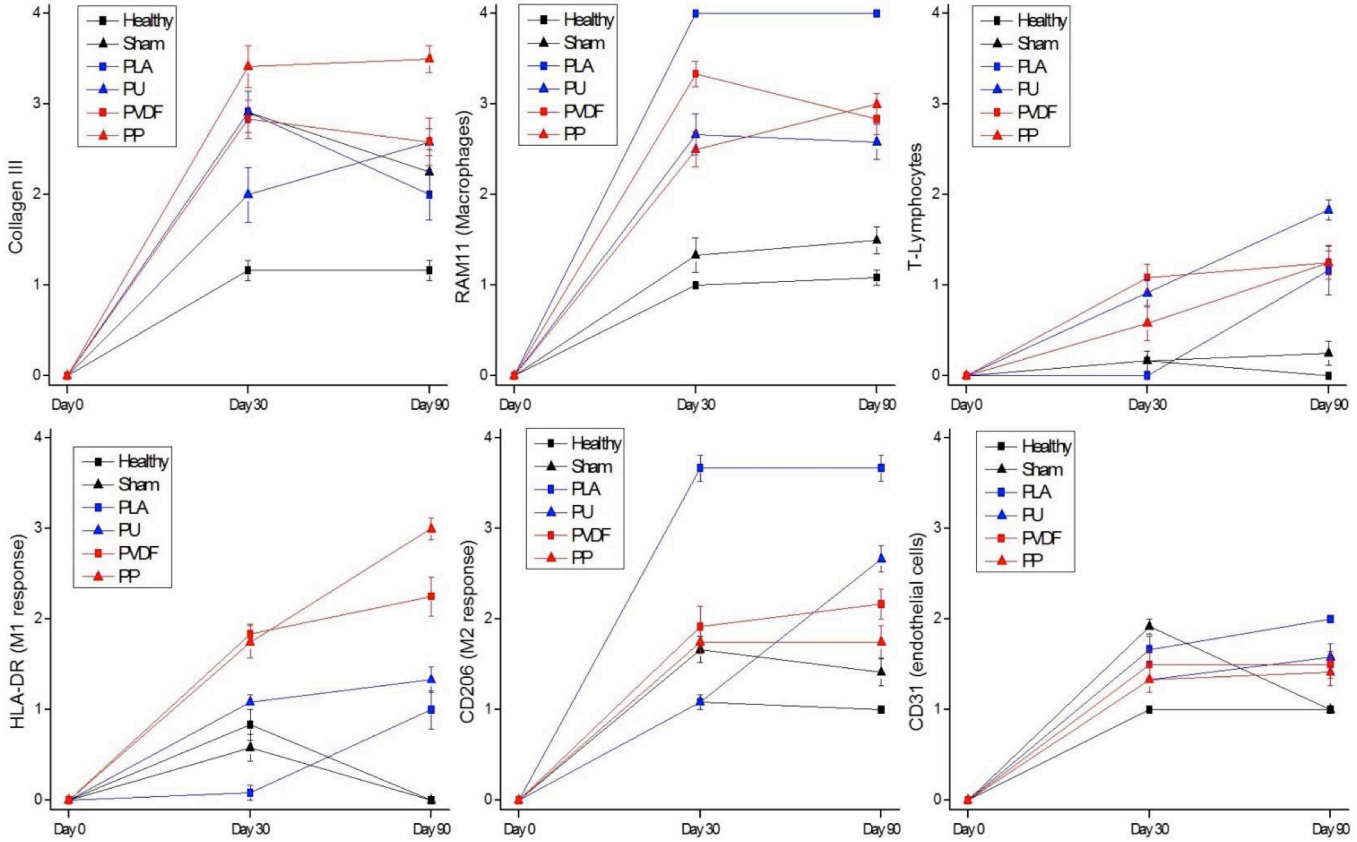


Appendix 4

Immunological outcomes at 90 days



Appendix 5



Mean blind scoring values for each immunohistochemistry data at 0, 30 and 90 days. Assessed as 0 = absence, 1 = mild presence, 2 = large presence, 3 = abundance, 4 =

Appendix 6

Individual Dunn's comparison test for blind scoring for immunohistochemistry data a

Collagen III	Significance	Ram 11	Significance	T-lymphcytes	Sign
Sham vs. PLA	ns	Sham vs. PLA	****	Sham vs. PLA	ns
Sham vs. PU	ns	Sham vs. PU	ns	Sham vs. PU	*
Sham vs. PVDF	ns	Sham vs. PVDF	***	Sham vs. PVDF	**
Sham vs. PPL	ns	Sham vs. PPL	ns	Sham vs. PPL	ns
Sham vs. Healthy	**	Sham vs. Healthy	ns	Sham vs. Healthy	ns
PLA vs. PU	ns	PLA vs. PU	ns	PLA vs. PU	**
PLA vs. PVDF	ns	PLA vs. PVDF	ns	PLA vs. PVDF	****
PLA vs. PPL	ns	PLA vs. PPL	*	PLA vs. PPL	ns
PLA vs. Healthy	**	PLA vs. Healthy	****	PLA vs. Healthy	ns
PU vs. PVDF	ns	PU vs. PVDF	ns	PU vs. PVDF	ns
PU vs. PPL	*	PU vs. PPL	ns	PU vs. PPL	ns
PU vs. Healthy	ns	PU vs. Healthy	**	PU vs. Healthy	*
PVDF vs. PPL	ns	PVDF vs. PPL	ns	PVDF vs. PPL	ns
PVDF vs. Healthy	**	PVDF vs. Healthy	****	PVDF vs. Healthy	**
PPL vs. Healthy	****	PPL vs. Healthy	*	PPL vs. Healthy	ns
HLA-DR	**Significance**	**CD206**	**Significance**	**CD31**	**Sign**
Sham vs. PLA	ns	Sham vs. PLA	**	Sham vs. PLA	ns
Sham vs. PU	ns	Sham vs. PU	ns	Sham vs. PU	ns
Sham vs. PVDF	***	Sham vs. PVDF	ns	Sham vs. PVDF	ns
Sham vs. PPL	**	Sham vs. PPL	ns	Sham vs. PPL	ns
Sham vs. Healthy	ns	Sham vs. Healthy	ns	Sham vs. Healthy	***
PLA vs. PU	****	PLA vs. PU	****	PLA vs. PU	ns
PLA vs. PVDF	****	PLA vs. PVDF	*	PLA vs. PVDF	ns
PLA vs. PPL	****	PLA vs. PPL	**	PLA vs. PPL	ns
PLA vs. Healthy	*	PLA vs. Healthy	****	PLA vs. Healthy	*
PU vs. PVDF	**	PU vs. PVDF	ns	PU vs. PVDF	ns
PU vs. PPL	**	PU vs. PPL	ns	PU vs. PPL	ns
PU vs. Healthy	ns	PU vs. Healthy	ns	PU vs. Healthy	ns
PVDF vs. PPL	ns	PVDF vs. PPL	ns	PVDF vs. PPL	ns
PVDF vs. Healthy	**	PVDF vs. Healthy	ns	PVDF vs. Healthy	ns
PPL vs. Healthy	**	PPL vs. Healthy	ns	PPL vs. Healthy	ns

ns – not significant

* - p<0.05

** - p<0.01

*** - p<0.001

**** - p<0.0001

Appendix 7

Individual Dunn's comparison test for blind scoring for immunohistochemistry data at 90 days.

Collagen III	Significance	Ram 11	Significance	T-lymphocytes	Significance
Sham vs. PLA	ns	Sham vs. PLA	****	Sham vs. PLA	ns
Sham vs. PU	ns	Sham vs. PU	ns	Sham vs. PU	****
Sham vs. PVDF	ns	Sham vs. PVDF	*	Sham vs. PVDF	*
Sham vs. PPL	*	Sham vs. PPL	**	Sham vs. PPL	*
Sham vs. Healthy	ns	Sham vs. Healthy	ns	Sham vs. Healthy	ns
PLA vs. PU	ns	PLA vs. PU	*	PLA vs. PU	ns
PLA vs. PVDF	ns	PLA vs. PVDF	ns	PLA vs. PVDF	ns
PLA vs. PPL	**	PLA vs. PPL	ns	PLA vs. PPL	ns
PLA vs. Healthy	ns	PLA vs. Healthy	****	PLA vs. Healthy	**
PU vs. PVDF	ns	PU vs. PVDF	ns	PU vs. PVDF	ns
PU vs. PPL	ns	PU vs. PPL	ns	PU vs. PPL	ns
PU vs. Healthy	**	PU vs. Healthy	*	PU vs. Healthy	****
PVDF vs. PPL	ns	PVDF vs. PPL	ns	PVDF vs. PPL	ns
PVDF vs. Healthy	**	PVDF vs. Healthy	**	PVDF vs. Healthy	**
PPL vs. Healthy	****	PPL vs. Healthy	***	PPL vs. Healthy	**
HLA-DR	**Significance**	**CD206**	**Significance**	**CD31**	**Significance**
Sham vs. PLA	ns	Sham vs. PLA	****	Sham vs. PLA	****
Sham vs. PU	*	Sham vs. PU	*	Sham vs. PU	ns
Sham vs. PVDF	****	Sham vs. PVDF	ns	Sham vs. PVDF	ns
Sham vs. PPL	****	Sham vs. PPL	ns	Sham vs. PPL	ns
Sham vs. Healthy	ns	Sham vs. Healthy	ns	Sham vs. Healthy	ns
PLA vs. PU	ns	PLA vs. PU	ns	PLA vs. PU	ns
PLA vs. PVDF	ns	PLA vs. PVDF	*	PLA vs. PVDF	ns
PLA vs. PPL	**	PLA vs. PPL	***	PLA vs. PPL	ns
PLA vs. Healthy	ns	PLA vs. Healthy	****	PLA vs. Healthy	****
PU vs. PVDF	ns	PU vs. PVDF	ns	PU vs. PVDF	ns
PU vs. PPL	ns	PU vs. PPL	ns	PU vs. PPL	ns
PU vs. Healthy	*	PU vs. Healthy	****	PU vs. Healthy	ns
PVDF vs. PPL	ns	PVDF vs. PPL	ns	PVDF vs. PPL	ns
PVDF vs. Healthy	****	PVDF vs. Healthy	*	PVDF vs. Healthy	ns
PPL vs. Healthy	****	PPL vs. Healthy	ns	PPL vs. Healthy	ns

ns – not significant

* - p<0.05

** - p<0.01

*** - p<0.001

**** - p<0.0001

References

[1] Abdel-Fattah, M., A. Familusi, S. Fielding, J. Ford, and S. Bhattacharya, Primary and repeat surgical treatment for female pelvic organ prolapse and incontinence in parous women in the UK: a register linkage study. BMJ Open 2011;1:e000206.

[2] Dumoulin C, Hunter KF, Moore K, Bradley CS, Burgio KL, Hagen S, Inamura M, Thakar R, Williams K, Chambers T, Conservative management for female urinary incontinence and pelvic organ prolapse review 2013: Summary of the 5th International Consultation on Incontinence. Neurourol Urodyn. 2016;35(1):15-20.

[3] Coyne, K. and C. Kelleher, Patient reported outcomes: the ICIQ and the state of the art. Neurourol Urodyn 2010;29:645-51.

[4] Milsom, I., Altman, D., Cartwright, R., Lapitan, M.C., Nelson, R., Sillen, U., Tikkinen, K. , Epidemiology of Urinary Incontinence (UI) and other Lower Urinary Tract Symptoms (LUTS), Pelvic Organ Prolapse (POP) and Anal Incontinence (AI), in Incontinence (5th International Consultation on Incontinence), P. Abrams, Cardozo, L., Khoury, S., Wein, A., Editor. 2013: Paris. p.15-107..

[5] Tennstedt, S.L., C.L. Link, W.D. Steers, and J.B. McKinlay, Prevalence ofand risk factors for urine leakage in a racially and ethnically diverse population of adults: the Boston Area Community Health (BACH) Survey. Am J Epidemiol 2008;167:390-9.

[6] Coyne, K.S., C.C. Sexton, C.L. Thompson, I. Milsom, D. Irwin, Z.S. Kopp, C.R. Chapple, S. Kaplan, A. Tubaro, L.P. Aiyer, and A.J. Wein, The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: results from the Epidemiology of LUTS (EpiLUTS) study. BJU Int 2009;104:352-60.

[7] Hannestad, Y.S., G. Rortveit, H. Sandvik, and S. Hunskaar, A community-based epidemiological survey of female urinary incontinence: the Norwegian EPINCONT study. Epidemiology of Incontinence in the County of Nord-Trondelag. J Clin Epidemiol 2000;53:1150-7.

[8] Lifford, K.L., M.K. Townsend, G.C. Curhan, N.M. Resnick, and F. Grodstein, The epidemiology of urinary incontinence in older women: incidence, progression, and remission. J Am Geriatr Soc 2008;56:1191-8.

[9] Minassian, V.A., W.F. Stewart, and A.G. Hirsch, Why do stress and urge incontinence co-occur much more often than expected? Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1429-40.

[10] Press, J.Z., M.C. Klein, J. Kaczorowski, R.M. Liston, and P. von Dadelszen, Does cesarean section reduce postpartum urinary incontinence? A systematic review. Birth 2007;34:228-37.

[11] Thom, D.H. and G. Rortveit, Prevalence of postpartum urinary incontinence: a systematic review. Acta Obstet Gynecol Scand 2010;89:1511-22.

[12] Herrmann, V., K. Scarpa, P.C. Palma, and C.Z. Riccetto, Stress urinary incontinence 3 years after pregnancy: correlation to mode of delivery and parity. Int Urogynecol J Pelvic Floor Dysfunct 2009;20:281-8.

[13] Hojberg, K.E., J.D. Salvig, N.A. Winslow, G. Lose, and N.J. Secher, Urinary incontinence: prevalence and risk factors at 16 weeks of gestation. Br J Obstet Gynaecol 1999;106:842-50.

[14] Thomas, T.M., K.R. Plymat, J. Blannin, and T.W. Meade, Prevalence of urinary incontinence. Br Med J 1980;281:1243-5.

[15] Rortveit, G., Y.S. Hannestad, A.K. Daltveit, and S. Hunskaar, Age- and type-

dependent effects of parity on urinary incontinence: the Norwegian EPINCONT study. Obstet Gynecol 2001;98:1004-10.
[16] Hunskaar, S., A systematic review of overweight and obesity as risk factors and targets for clinical intervention for urinary incontinence in women. Neurourol Urodyn 2008;27:749-57.
[17] Subak, L.L., H.E. Richter, and S. Hunskaar, Obesity and urinary incontinence: epidemiology and clinical research update. J Urol 2009;182:S2-7.
[18] Richter, H.E., J.M. Creasman, D.L. Myers, T.L. Wheeler, K.L. Burgio, L.L. Subak, D. Program to Reduce Incontinence by, and G. Exercise Research, Urodynamic characterization of obese women with urinary incontinence undergoing a weight loss program: the Program to Reduce Incontinence by Diet and Exercise (PRIDE) trial. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1653-8.
[19] Deitel, M., E. Stone, H.A. Kassam, E.J. Wilk, and D.J. Sutherland, Gynecologic-obstetric changes after loss of massive excess weight following bariatric surgery. J Am Coll Nutr 1988;7:147-53.
[20] Hannestad, Y.S., R.T. Lie, G. Rortveit, and S. Hunskaar, Familial risk of urinary incontinence in women: population based cross sectional study. BMJ 2004;329:889-91.
[21] Wennberg, A.L., D. Altman, C. Lundholm, A. Klint, A. Iliadou, R. Peeker, M. Fall, N.L. Pedersen, and I. Milsom, Genetic influences are important for most but not all lower urinary tract symptoms: a population-based survey in a cohort of adult Swedish twins. Eur Urol 2011;59:1032-8.
[22] McKenzie, P., J. Rohozinski, and G. Badlani, Genetic influences on stress urinary incontinence. Current Opinion in Urology 2010;20:291-295.
[23] Luber, K.M., S. Boero, and J.Y. Choe, The demographics of pelvic floor disorders: current observations and future projections. Am J Obstet Gynecol 2001;184:1496-501; discussion 1501-3.
[24] Turner, D.A., C. Shaw, C.W. McGrother, H.M. Dallosso, N.J. Cooper, and M.R.C.I. Team, The cost of clinically significant urinary storage symptoms for community dwelling adults in the UK. BJU Int 2004;93:1246-52.
[25] Wilson, L., J.S. Brown, G.P. Shin, K.O. Luc, and L.L. Subak, Annual direct cost of urinary incontinence. Obstet Gynecol 2001;98:398-406.
[26] Birnbaum, H., S. Leong, and A. Kabra, Lifetime medical costs for women: cardiovascular disease, diabetes, and stress urinary incontinence. Womens Health Issues 2003;13:204-13.
[27] Coyne, K.S., A.J. Wein, A. Tubaro, C.C. Sexton, C.L. Thompson, Z.S. Kopp, and L.P. Aiyer, The burden of lower urinary tract symptoms: evaluating the effect of LUTS on health-related quality of life, anxiety and depression: EpiLUTS. BJU Int 2009;103 Suppl 3:4-11.
[28] Coyne, K.S., Z. Zhou, C. Thompson, and E. Versi, The impact on health-related quality of life of stress, urge and mixed urinary incontinence. BJU Int 2003;92:731-5.
[29] Barber, M.D. and C. Maher, Epidemiology and outcome assessment of pelvic organ prolapse. Int Urogynecol J 2013;24:1783-90.
[30] Swift, S., P. Woodman, A. O'Boyle, M. Kahn, M. Valley, D. Bland, W. Wang, and J. Schaffer, Pelvic Organ Support Study (POSST): the distribution, clinical definition, and epidemiologic condition of pelvic organ support defects. Am J Obstet Gynecol 2005;192:795-806.

[31] Baden, W.F. and T.A. Walker, Physical diagnosis in the evaluation of vaginal relaxation. Clin Obstet Gynecol 1972;15:1055-69.
[32] Porges, R.F., A Practical System of Diagnosis and Classification of Pelvic Relaxations. Surg Gynecol Obstet 1963;117:769-73.
[33] Bump, R.C., A. Mattiasson, K. Bo, L.P. Brubaker, J.O. DeLancey, P. Klarskov, B.L. Shull, and A.R. Smith, The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. Am J Obstet Gynecol 1996;175:10-7.
[34] Rortveit, G., J.S. Brown, D.H. Thom, S.K. Van Den Eeden, J.M. Creasman, and L.L. Subak, Symptomatic pelvic organ prolapse: prevalence and risk factors in a population-based, racially diverse cohort. Obstet Gynecol 2007;109:1396-403.
[35] Lawrence, J.M., E.S. Lukacz, C.W. Nager, J.W. Hsu, and K.M. Luber, Prevalence and co-occurrence of pelvic floor disorders in community-dwelling women. Obstet Gynecol 2008;111:678-85.
[36] Handa, V.L., E. Garrett, S. Hendrix, E. Gold, and J. Robbins, Progression and remission of pelvic organ prolapse: a longitudinal study of menopausal women. Am J Obstet Gynecol 2004;190:27-32.
[37] Hendrix, S.L., A. Clark, I. Nygaard, A. Aragaki, V. Barnabei, and A. McTiernan, Pelvic organ prolapse in the Women's Health Initiative: gravity and gravidity. Am J Obstet Gynecol 2002;186:1160-6.
[38] Nygaard, I., C. Bradley, D. Brandt, and I. Women's Health, Pelvic organ prolapse in older women: prevalence and risk factors. Obstet Gynecol 2004;104:489-97.
[39] Mant, J., R. Painter, and M. Vessey, Epidemiology of genital prolapse: observations from the Oxford Family Planning Association Study. Br J Obstet Gynaecol 1997;104:579-85.
[40] Olsen, A.L., V.J. Smith, J.O. Bergstrom, J.C. Colling, and A.L. Clark, Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol 1997;89:501-6.
[41] Altman, D., J. Zetterstrom, I. Schultz, J. Nordenstam, F. Hjern, A. Lopez, and A. Mellgren, Pelvic organ prolapse and urinary incontinence in women with surgically managed rectal prolapse: a population-based case-control study. Dis Colon Rectum 2006;49:28-35.
[42] Moalli, P.A., S. Jones Ivy, L.A. Meyn, and H.M. Zyczynski, Risk factors associated with pelvic floor disorders in women undergoing surgical repair. Obstet Gynecol 2003;101:869-74.
[43] Wiskind, A.K., S.M. Creighton, and S.L. Stanton, The incidence of genital prolapse after the Burch colposuspension. Am J Obstet Gynecol 1992;167:399-404; discussion 404-5.
[44] Denman, M.A., W.T. Gregory, S.H. Boyles, V. Smith, S.R. Edwards, and A.L. Clark, Reoperation 10 years after surgically managed pelvic organ prolapse and urinary incontinence. Am J Obstet Gynecol 2008;198:555 e1-5.
[45] Chiaffarino, F., L. Chatenoud, M. Dindelli, M. Meschia, A. Buonaguidi, F. Amicarelli, M. Surace, E. Bertola, E. Di Cintio, and F. Parazzini, Reproductive factors, family history, occupation and risk of urogenital prolapse. Eur J Obstet Gynecol Reprod Biol 1999;82:63-7.
[46] MacLennan, A.H., A.W. Taylor, D.H. Wilson, and D. Wilson, The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of

delivery. BJOG 2000;107:1460-70.
[47] Leijonhufvud, A., C. Lundholm, S. Cnattingius, F. Granath, E. Andolf, and D. Altman, Risks of stress urinary incontinence and pelvic organ prolapse surgery in relation to mode of childbirth. Am J Obstet Gynecol 2011;204:70 e1-7.
[48] O'Boyle, A.L., P.J. Woodman, J.D. O'Boyle, G.D. Davis, and S.E. Swift, Pelvic organ support in nulliparous pregnant and nonpregnant women: a casecontrol study. Am J Obstet Gynecol 2002;187:99-102.
[49] Washington, B.B., E.A. Erekson, N.C. Kassis, and D.L. Myers, The association between obesity and stage II or greater prolapse. Am J Obstet Gynecol 2010;202:503 e1-4.
[50] Carley, M.E. and J. Schaffer, Urinary incontinence and pelvic organ prolapse in women with Marfan or Ehlers Danlos syndrome. Am J Obstet Gynecol 2000;182:1021-3.
[51] McLennan, M.T., J.K. Harris, B. Kariuki, and S. Meyer, Family history as a risk factor for pelvic organ prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1063-9.
[52] Slieker-ten Hove, M.C., A.L. Pool-Goudzwaard, M.J. Eijkemans, R.P. Steegers-Theunissen, C.W. Burger, and M.E. Vierhout, Symptomatic pelvic organ prolapse and possible risk factors in a general population. Am J Obstet Gynecol 2009;200:184 e1-7.
[53] Jack, G.S., G. Nikolova, E. Vilain, S. Raz, and L.V. Rodriguez, Familial transmission of genitovaginal prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2006;17:498-501.
[54] Subak, L.L., L.E. Waetjen, S. van den Eeden, D.H. Thom, E. Vittinghoff, and J.S. Brown, Cost of pelvic organ prolapse surgery in the United States. Obstet Gynecol 2001;98:646-51.
[55] Subramanian, D., K. Szwarcensztein, J.A. Mauskopf, and M.C. Slack, Rate, type, and cost of pelvic organ prolapse surgery in Germany, France, and England. Eur J Obstet Gynecol Reprod Biol 2009;144:177-81.
[56] DeLancey, J.O., Structural support of the urethra as it relates to stress urinary incontinence: the hammock hypothesis. Am J Obstet Gynecol 1994;170:1713-20; discussion 1720-3.
[57] Rud, T., K.E. Andersson, M. Asmussen, A. Hunting, and U. Ulmsten, Factors maintaining the intraurethral pressure in women. Invest Urol 1980;17:343-7.
[58] Kayigil, O., S. Iftekhar Ahmed, and A. Metin, The coexistence of intrinsic sphincter deficiency with type II stress incontinence. J Urol 1999;162:1365-6.
[59] Enhorning, G., Simultaneous recording of intravesical and intra-urethral pressure. A study on urethral closure in normal and stress incontinent women. Acta Chir Scand Suppl 1961;Suppl 276:1-68.
[60] Constantinou, C.E. and D.E. Govan, Spatial distribution and timing of transmitted and reflexly generated urethral pressures in healthy women. J Urol 1982;127:964-9.
[61] Greenwald, S.W., J.R. Thornbury, and L.J. Dunn, Cystourethrography as a diagnostic aid in stress incontinence. An evaluation. Obstet Gynecol 1967;29:324-7.
[62] Petros, P.E. and U.I. Ulmsten, An integral theory of female urinary incontinence. Experimental and clinical considerations. Acta Obstet Gynecol Scand Suppl 1990;153:7-31.
[63] McGuire, E.J., Urodynamic findings in patients after failure of stress

incontinence operations. Prog Clin Biol Res 1981;78:351-60.
[64] McGuire, E.J., B. Lytton, E.I. Kohorn, and V. Pepe, The value of urodynamic testing in stress urinary incontinence. J Urol 1980;124:256-8.
[65] McGuire, E.J., B. Lytton, V. Pepe, and E.I. Kohorn, Stress Urinary Incontinence. Obstet Gynecol 1976;47:255-64.
[66] Blaivas, J.G. and C.A. Olsson, Stress incontinence: classification and surgical approach. J Urol 1988;139:727-31.
[67] Sand, P.K., L.W. Bowen, R. Panganiban, and D.R. Ostergard, The low pressure urethra as a factor in failed retropubic urethropexy. Obstet Gynecol 1987;69:399-402.
[68] Betson, L.H., G. Siddiqui, and N.N. Bhatia, Intrinsic urethral sphincteric deficiency: critical analysis of various diagnostic modalities. Curr Opin Obstet Gynecol 2003;15:411-7.
[69] McGuire, E.J., C.C. Fitzpatrick, J. Wan, D. Bloom, J. Sanvordenker, M. Ritchey, and E.A. Gormley, Clinical assessment of urethral sphincter function. J Urol 1993;150:1452-4.
[70] Thubert, T., X. Deffieux, M. Jousse, A. Guinet-Lacoste, S.S. Ismael, and G. Amarenco, Posterior vaginal wall pull down maneuver: a clinical test to diagnose intrinsic sphincter deficiency in women suffering from genuine urinary stress incontinence. Int J Urol 2013;20:1124-9.
[71] Gilpin, S.A., J.A. Gosling, A.R. Smith, and D.W. Warrell, The pathogenesis of genitourinary prolapse and stress incontinence of urine. A histological and histochemical study. Br J Obstet Gynaecol 1989;96:15-23.
[72] Dietz, H.P., A.V. Franco, K.L. Shek, and A. Kirby, Avulsion injury and levator hiatal ballooning: two independent risk factors for prolapse? An observational study. Acta Obstet Gynecol Scand 2012;91:211-4.
[73] van Delft, K., A. Sultan, R. Thakar, N. Schwertner-Tiepelmann, and K. Kluivers, The relationship between postpartum levator ani muscle avulsion and signs and symptoms of pelvic floor dysfunction. BJOG 2014;.
[74] DeLancey, J.O., D.M. Morgan, D.E. Fenner, R. Kearney, K. Guire, J.M. Miller, H. Hussain, W. Umek, Y. Hsu, and J.A. Ashton-Miller, Comparison of levator ani muscle defects and function in women with and without pelvic organ prolapse. Obstet Gynecol 2007;109:295-302.
[75] Morris, V.C., M.P. Murray, J.O. Delancey, and J.A. Ashton-Miller, A comparison of the effect of age on levator ani and obturator internus muscle cross-sectional areas and volumes in nulliparous women. Neurourol Urodyn 2012;31:481-6.
[76] Delancey, J.O. and W.W. Hurd, Size of the urogenital hiatus in the levator ani muscles in normal women and women with pelvic organ prolapse. Obstet Gynecol 1998;91:364-8.
[77] Paramore, R., *The Statics of the Female Pelvic Viscera*. 1918, London: H.K. lewis..
[78] Tulikangas, P.K., Defect theory of pelvic organ prolapse. Clin Obstet Gynecol 2005;48:662-7.
[79] Richardson, A.C., J.B. Lyon, and N.L. Williams, A new look at pelvic relaxation. Am J Obstet Gynecol 1976;126:568-73.
[80] Calleja-Agius, J. and M.P. Brincat, Urogenital atrophy. Climacteric 2009;12:279-85.
[81] Badiou, W., G. Granier, P.J. Bousquet, X. Monrozies, P. Mares, and R. de

Tayrac, Comparative histological analysis of anterior vaginal wall in women with pelvic organ prolapse or control subjects. A pilot study. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:723-9.
[82] Boreham, M.K., C.Y. Wai, R.T. Miller, J.I. Schaffer, and R.A. Word, Morphometric analysis of smooth muscle in the anterior vaginal wall of women with pelvic organ prolapse. Am J Obstet Gynecol 2002;187:56-63.
[83] Boreham, M.K., C.Y. Wai, R.T. Miller, J.I. Schaffer, and R.A. Word, Morphometric properties of the posterior vaginal wall in women with pelvic organ prolapse. Am J Obstet Gynecol 2002;187:1501-8; discussion 1508-9.
[84] Takacs, P., M. Gualtieri, M. Nassiri, K. Candiotti, and C.A. Medina, Vaginal smooth muscle cell apoptosis is increased in women with pelvic organ prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1559-64.
[85] Kannan, K., A. McConnell, M. McLeod, and A. Rane, Microscopic alterations of vaginal tissue in women with pelvic organ prolapse. J Obstet Gynaecol 2011;31:250-3.
[86] Abramowitch, S.D., A. Feola, Z. Jallah, and P.A. Moalli, Tissue mechanics, animal models, and pelvic organ prolapse: a review. Eur J Obstet Gynecol Reprod Biol 2009;144 Suppl 1:S146-58.
[87] Ramachandran, G.N. and G. Kartha, Structure of collagen. Nature 1955;176:593-5.
[88] Sherman, V.R., W. Yang, and M.A. Meyers, The materials science of collagen. J Mech Behav Biomed Mater 2015;52:22-50.
[89] Kerkhof, M.H., L. Hendriks, and H.A. Brolmann, Changes in connective tissue in patients with pelvic organ prolapse--a review of the current literature. Int Urogynecol J Pelvic Floor Dysfunct 2009;20:461-74.
[90] Bailey, A.J., Molecular mechanisms of ageing in connective tissues. Mech Ageing Dev 2001;122:735-55.
[91] Gelse, K., E. Poschl, and T. Aigner, Collagens--structure, function, and biosynthesis. Adv Drug Deliv Rev 2003;55:1531-46.
[92] Jarvelainen, H., P. Puolakkainen, S. Pakkanen, E.L. Brown, M. Hook, R.V. Iozzo, E.H. Sage, and T.N. Wight, A role for decorin in cutaneous wound healing and angiogenesis. Wound Repair Regen 2006;14:443-52.
[93] Svensson, L., A. Aszodi, F.P. Reinholt, R. Fassler, D. Heinegard, and A. Oldberg, Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. J Biol Chem 1999;274:9636-47.
[94] Chakravarti, S., T. Magnuson, J.H. Lass, K.J. Jepsen, C. LaMantia, and H. Carroll, Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. J Cell Biol 1998;141:1277-86.
[95] Campeau, L., I. Gorbachinsky, G.H. Badlani, and K.E. Andersson, Pelvic floor disorders: linking genetic risk factors to biochemical changes. BJU Int 2011;108:1240-7.
[96] Green, E.M., J.C. Mansfield, J.S. Bell, and C.P. Winlove, The structure and micromechanics of elastic tissue. Interface Focus 2014;4:20130058.
[97] Christiano, A.M. and J. Uitto, Molecular pathology of the elastic fibers. J Invest Dermatol 1994;103:53S-57S.
[98] Woessner, J.F. and T.H. Brewer, Formation and Breakdown of Collagen and Elastin in the Human Uterus during Pregnancy and Post-Partum Involution. Biochem J 1963;89:75-82.

[99] Chen, B.H., Y. Wen, H. Li, and M.L. Polan, Collagen metabolism and turnover in women with stress urinary incontinence and pelvic prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2002;13:80-7; discussion 87.
[100] Moalli, P.A., S.H. Shand, H.M. Zyczynski, S.C. Gordy, and L.A. Meyn, Remodeling of vaginal connective tissue in patients with prolapse. Obstet Gynecol 2005;106:953-63.
[101] Goepel, C., L. Hefler, H.D. Methfessel, and H. Koelbl, Periurethral connective tissue status of postmenopausal women with genital prolapse with and without stress incontinence. Acta Obstet Gynecol Scand 2003;82:659-64.
[102] Ulmsten, U., G. Ekman, G. Giertz, and A. Malmstrom, Different biochemical composition of connective tissue in continent and stress incontinent women. Acta Obstet Gynecol Scand 1987;66:455-7.
[103] Takano, C.C., M.J. Girao, M.G. Sartori, R.A. Castro, R.M. Arruda, M.J. Simoes, E.C. Baracat, and G. Rodrigues de Lima, Analysis of collagen in parametrium and vaginal apex of women with and without uterine prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2002;13:342-5; discussion 345.
[104] Aznal, S.S., Meng, F.G., Nalliah, S, Tay, A., Chinniah, K., Jamil, M.F. , Biochemical evaluation of the supporting structure of the pelvic organs in selected numbers of premenopausal and postmenopausal Malaysian women. Indian Journal of Pathology and Microbiology 2012;55:450-455.
[105] Kokcu, A., F. Yanik, M. Cetinkaya, T. Alper, B. Kandemir, and E. Malatyalioglu, Histopathological evaluation of the connective tissue of the vaginal fascia and the uterine ligaments in women with and without pelvic relaxation. Arch Gynecol Obstet 2002;266:75-8.
[106] Merkel, J.R., B.R. DiPaolo, G.G. Hallock, and D.C. Rice, Type I and type III collagen content of healing wounds in fetal and adult rats. Proc Soc Exp Biol Med 1988;187:493-7.
[107] Ewies, A.A., F. Al-Azzawi, and J. Thompson, Changes in extracellular matrix proteins in the cardinal ligaments of post-menopausal women with or without prolapse: a computerized immunohistomorphometric analysis. Hum Reprod 2003;18:2189-95.
[108] Gabriel, B., D. Denschlag, H. Gobel, C. Fittkow, M. Werner, G. Gitsch, and D. Watermann, Uterosacral ligament in postmenopausal women with or without pelvic organ prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2005;16:475-9.
[109] De Landsheere, L., C. Munaut, B. Nusgens, C. Maillard, C. Rubod, M. Nisolle, M. Cosson, and J.M. Foidart, Histology of the vaginal wall in women with pelvic organ prolapse: a literature review. Int Urogynecol J 2013;24:2011-20.
[110] Makinen, J., V.M. Kahari, K.O. Soderstrom, E. Vuorio, and T. Hirvonen, Collagen synthesis in the vaginal connective tissue of patients with and without uterine prolapse. Eur J Obstet Gynecol Reprod Biol 1987;24:319-25.
[111] Edwall, L., K. Carlstrom, and A. Fianu Jonasson, Markers of collagen synthesis and degradation in urogenital tissue and serum from women with and without uterovaginal prolapse. Mol Hum Reprod 2008;14:193-7.
[112] Suzme, R., O. Yalcin, F. Gurdol, F. Gungor, and A. Bilir, Connective tissue alterations in women with pelvic organ prolapse and urinary incontinence. Acta Obstet Gynecol Scand 2007;86:882-8.
[113] Jackson, S.R., N.C. Avery, J.F. Tarlton, S.D. Eckford, P. Abrams, and A.J. Bailey, Changes in metabolism of collagen in genitourinary prolapse. Lancet 1996;347:1658-61.

[114] McIntosh, L.J., V.T. Mallett, J.D. Frahm, D.A. Richardson, and M.I. Evans, Gynecologic disorders in women with Ehlers-Danlos syndrome. J Soc Gynecol Investig 1995;2:559-64.
[115] Rinne, K.M. and P.P. Kirkinen, What predisposes young women to genital prolapse? Eur J Obstet Gynecol Reprod Biol 1999;84:23-5.
[116] Deval, B., A. Rafii, S. Poilpot, N. Aflack, and M. Levardon, [Prolapse in the young woman: study of risk factors]. Gynecol Obstet Fertil 2002;30:673-6.
[117] Soderberg, M.W., C. Falconer, B. Bystrom, A. Malmstrom, and G. Ekman, Young women with genital prolapse have a low collagen concentration. Acta Obstet Gynecol Scand 2004;83:1193-8.
[118] Yamamoto, K., M. Yamamoto, K. Akazawa, S. Tajima, H. Wakimoto, and M. Aoyagi, Decrease in elastin gene expression and protein synthesis in fibroblasts derived from cardinal ligaments of patients with prolapsus uteri. Cell Biol Int 1997;21:605-11.
[119] Chen, B., Y. Wen, and M.L. Polan, Elastolytic activity in women with stress urinary incontinence and pelvic organ prolapse. Neurourol Urodyn 2004;23:119-26.
[120] Karam, J.A., D.V. Vazquez, V.K. Lin, and P.E. Zimmern, Elastin expression and elastic fibre width in the anterior vaginal wall of postmenopausal women with and without prolapse. BJU Int 2007;100:346-50.
[121] Shah, D.K., Kushner, L., Rao, S.K., et al. Elastase Activity in Plasma: Screening Tool for Stress Urinary Incontinence, in AUA. 2002: Orlando, FL.
[122] Poncet, S., S. Meyer, C. Richard, J.D. Aubert, and L. Juillerat-Jeanneret, The expression and function of the endothelin system in contractile properties ofvaginal myofibroblasts of women with uterovaginal prolapse. Am J Obstet Gynecol 2005;192:426-32.
[123] Meyer, S., C. Achtari, P. Hohlfeld, and L. Juillerat-Jeanneret, The contractile properties of vaginal myofibroblasts: is the myofibroblasts contraction force test a valuable indication of future prolapse development? Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1399-403.
[124] Ewies, A.A., M. Elshafie, J. Li, A. Stanley, J. Thompson, J. Styles, I. White, and F. Al-Azzawi, Changes in transcription profile and cytoskeleton morphology in pelvic ligament fibroblasts in response to stretch: the effects of estradiol and levormeloxifene. Mol Hum Reprod 2008;14:127-35.
[125] Wen, Y., Y.Y. Zhao, M.L. Polan, and B. Chen, Effect of relaxin on TGF-beta1 expression in cultured vaginal fibroblasts from women with stress urinary incontinence. Reprod Sci 2008;15:312-20.
[126] Clark, A.L., O.D. Slayden, K. Hettrich, and R.M. Brenner, Estrogen increases collagen I and III mRNA expression in the pelvic support tissues of the rhesus macaque. Am J Obstet Gynecol 2005;192:1523-9.
[127] Rizk, D.E., H.A. Hassan, A.H. Al-Marzouqi, G.A. Ramadan, S.S. Al-Kedrah, S.A. Daoud, and M.A. Fahim, Combined estrogen and ghrelin administration restores number of blood vessels and collagen type I/III ratio in the urethral and anal canal submucosa of old ovariectomized rats. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:547-52.
[128] Chen, B., Y. Wen, H. Wang, and M.L. Polan, Differences in estrogen modulation of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-1 expression in cultured fibroblasts from continent and incontinent women. Am J Obstet Gynecol 2003;189:59-65.
[129] Moehrer, B., A. Hextall, and S. Jackson, Oestrogens for urinary incontinence in

women. Cochrane Database Syst Rev 2003;CD001405.
[130] Zong, W., H.M. Zyczynski, L.A. Meyn, S.C. Gordy, and P.A. Moalli, Regulation of MMP-1 by sex steroid hormones in fibroblasts derived from the female pelvic floor. Am J Obstet Gynecol 2007;196:349 e1-11.
[131] Zong, W., L.A. Meyn, and P.A. Moalli, The amount and activity of active matrix metalloproteinase 13 is suppressed by estradiol and progesterone in human pelvic floor fibroblasts. Biol Reprod 2009;80:367-74.
[132] Moalli, P.A., L.C. Talarico, V.W. Sung, W.L. Klingensmith, S.H. Shand, L.A. Meyn, and S.C. Watkins, Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis. Am J Obstet Gynecol 2004;190:620-7.
[133] Ahn, K.H., T. Kim, J.Y. Hur, S.H. Kim, K.W. Lee, and Y.T. Kim, Relationship between serum estradiol and follicle-stimulating hormone levels and urodynamic results in women with stress urinary incontinence. Int Urogynecol J 2011;22:731-7.
[134] Baragi, R.V., J.O. Delancey, R. Caspari, D.H. Howard, and J.A. Ashton-Miller, Differences in pelvic floor area between African American and European American women. Am J Obstet Gynecol 2002;187:111-5.
[135] Ashton-Miller, J.A. and J.O. Delancey, On the biomechanics of vaginal birth and common sequelae. Annu Rev Biomed Eng 2009;11:163-76.
[136] Lee, T.Q. and S.L. Woo, A new method for determining cross-sectional shape and area of soft tissues. J Biomech Eng 1988;110:110-4.
[137] Moon, D.K., S.D. Abramowitch, and S.L. Woo, The development and validation of a charge-coupled device laser reflectance system to measure the complex cross-sectional shape and area of soft tissues. J Biomech 2006;39:3071-5.
[138] Sacks, M.S., Biaxial mechanical evaluation of planar biological materials. Journal of Elasticity 2000;61:199-246.
[139] Ettema, G.J., J.T. Goh, and M.R. Forwood, A new method to measure elastic properties of plastic-viscoelastic connective tissue. Med Eng Phys 1998;20:308-14.
[140] Fu, X., Siltberg, H., Johnson P., Ulmsten, U., Viscoelastic properties and muscular function of the human anterior vaginal wall Int Urogynecol J 1995;6:229-234.
[141] Pena, E., B. Calvo, M.A. Martinez, P. Martins, T. Mascarenhas, R.M. Jorge, A. Ferreira, and M. Doblare, Experimental study and constitutive modeling of the viscoelastic mechanical properties of the human prolapsed vaginal tissue. Biomech Model Mechanobiol 2010;9:35-44.
[142] Robi, K., Jakob, N., Matevz, K., Matjaz, V. , The Physiology of Sports Injuries and Repair Processes, in Current Issues in Sports and Exercise Medicine, M. Hamlin, Editor. 2013, In-Tech..
[143] Diani, J., B. Fayolle, and P. Gilormini, A review on the Mullins effect. European Polymer Journal 2009;45:601-612.
[144] Lei, L., Y. Song, and R. Chen, Biomechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women. Int Urogynecol J Pelvic Floor Dysfunct 2007;18:603-7.
[145] Zimmern, P.E., R.C. Eberhart, and A. Bhatt, Methodology for biomechanical testing of fresh anterior wall vaginal samples from postmenopausal women undergoing cystocele repair. Neurourol Urodyn 2009;28:325-9.
[146] Martins, P., A. Lopes Silva-Filho, A.M. Rodrigues Maciel da Fonseca, A. Santos,

L. Santos, T. Mascarenhas, R.M. Natal Jorge, and A.J. Ferreira, Biomechanical properties of vaginal tissue in women with pelvic organ prolapse. Gynecol Obstet Invest 2013;75:85-92.
[147] Jean-Charles, C., C. Rubod, M. Brieu, M. Boukerrou, J. Fasel, and M. Cosson, Biomechanical properties of prolapsed or non-prolapsed vaginal tissue: impact on genital prolapse surgery. Int Urogynecol J 2010;21:1535-8.
[148] Rubod, C., M. Boukerrou, M. Brieu, C. Jean-Charles, P. Dubois, and M. Cosson, Biomechanical properties of vaginal tissue: preliminary results. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:811-6.
[149] Goh, J.T., Biomechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women. Int Urogynecol J Pelvic Floor Dysfunct 2002;13:76-9; discussion 79.
[150] Moalli, P.A., K.M. Debes, L.A. Meyn, N.S. Howden, and S.D. Abramowitch, Hormones restore biomechanical properties of the vagina and supportive tissues after surgical menopause in young rats. Am J Obstet Gynecol 2008;199:161 e1-8.
[151] Gilchrist, A.S., A. Gupta, R.C. Eberhart, and P.E. Zimmern, Do biomechanical properties of anterior vaginal wall prolapse tissue predict outcome of surgical repair? J Urol 2010;183:1069-73.
[152] Turner, D.A., C. Shaw, C.W. McGrother, H.M. Dallosso, and N.J. Cooper, The cost of clinically significant urinary storage symptoms for community dwelling adults in the UK. BJU Int 2004;93:1246-52.
[153] Petros, P.E. and U.I. Ulmsten, The combined intravaginal sling and tuck operation. An ambulatory procedure for cure of stress and urge incontinence. Acta Obstet Gynecol Scand Suppl 1990;153:53-9.
[154] McGuire, E.J. and B. Lytton, Pubovaginal sling procedure for stress incontinence. J Urol 1978;119:82-4.
[155] Ward, K., P. Hilton, K. United, and G. Ireland Tension-free Vaginal Tape Trial, Prospective multicentre randomised trial of tension-free vaginal tape and colposuspension as primary treatment for stress incontinence. BMJ 2002;325:67.
[156] Ford, A.A., L. Rogerson, J.D. Cody, and J. Ogah, Mid-urethral sling operations for stress urinary incontinence in women. Cochrane Database Syst Rev 2015;7:CD006375.
[157] Novara, G., W. Artibani, M.D. Barber, C.R. Chapple, E. Costantini, V. Ficarra, P. Hilton, C.G. Nilsson, and D. Waltregny, Updated systematic review and meta-analysis of the comparative data on colposuspensions, pubovaginal slings, and midurethral tapes in the surgical treatment of female stress urinary incontinence. Eur Urol 2010;58:218-38.
[158] Jia, X., C. Glazener, G. Mowatt, G. MacLennan, C. Bain, C. Fraser, and J. Burr, Efficacy and safety of using mesh or grafts in surgery for anterior and/or posterior vaginal wall prolapse: systematic review and meta-analysis. BJOG 2008;115:1350-61.
[159] Wasserberg, N., M. Haney, P. Petrone, M. Ritter, C. Emami, J. Rosca, K. Siegmund, and H.S. Kaufman, Morbid obesity adversely impacts pelvic floor function in females seeking attention for weight loss surgery. Dis Colon Rectum 2007;50:2096-103.
[160] Kegel, A.H., Progressive resistance exercise in the functional restoration of the perineal muscles. Am J Obstet Gynecol 1948;56:238-48.

[161] Hagen, S., D. Stark, C. Glazener, S. Dickson, S. Barry, A. Elders, H. Frawley, M.P. Galea, J. Logan, A. McDonald, G. McPherson, K.H. Moore, J. Norrie, A. Walker, D. Wilson, and P.T. Collaborators, Individualised pelvic floor muscle training in women with pelvic organ prolapse (POPPY): a multicentre randomised controlled trial. Lancet 2014;383:796-806.
[162] Piya-Anant, M., S. Therasakvichya, C. Leelaphatanadit, and K. Techatrisak, Integrated health research program for the Thai elderly: prevalence of genital prolapse and effectiveness of pelvic floor exercise to prevent worsening of genital prolapse in elderly women. J Med Assoc Thai 2003;86:509-15.
[163] Thubert, T., X. Deffieux, V. Letouzey, and J.F. Hermieu, [Obesity and urogynecology: a systematic review]. Prog Urol 2012;22:445-53.
[164] Kapoor, D.S., R. Thakar, A.H. Sultan, and R. Oliver, Conservative versus surgical management of prolapse: what dictates patient choice? Int Urogynecol J Pelvic Floor Dysfunct 2009;20:1157-61.
[165] Cundiff, G.W., A.C. Weidner, A.G. Visco, R.C. Bump, and W.A. Addison, A survey of pessary use by members of the American urogynecologic society. Obstet Gynecol 2000;95:931-5.
[166] Wu, V., S.A. Farrell, T.F. Baskett, and G. Flowerdew, A simplified protocol for pessary management. Obstet Gynecol 1997;90:990-4.
[167] Lamers, B.H., B.M. Broekman, and A.L. Milani, Pessary treatment for pelvic organ prolapse and health-related quality of life: a review. Int Urogynecol J 2011;22:637-44.
[168] Lin, H.H., B.C. Sheu, M.C. Lo, and S.C. Huang, Comparison of treatment outcomes for imipramine for female genuine stress incontinence. Br J Obstet Gynaecol 1999;106:1089-92.
[169] Norton, P.A., N.R. Zinner, I. Yalcin, R.C. Bump, and G. Duloxetine Urinary Incontinence Study, Duloxetine versus placebo in the treatment of stress urinary incontinence. Am J Obstet Gynecol 2002;187:40-8.
[170] Koelbl H, Nitti V, Baessler K, Salvatore S, Sultan A, Yamaguchi O, Pathophysiology of Urinary Incontinence, Faecal Incontinence and Pelvic organ prolapse, in Incontinence. 2009, Abrams P, Khoury S, Wein A. Health publication: Paris 2009;255-330.
[171] Rahn, D.D., M.M. Good, S.M. Roshanravan, H. Shi, J.I. Schaffer, R.J. Singh, and R.A. Word, Effects of Preoperative Local Estrogen in Postmenopausal Women with Prolapse: a Randomized Trial. J Clin Endocrinol Metab 2014;jc20141216.
[172] Hamer, M.A., K. Kallen, J. Lidfeldt, G. Samsioe, and P. Teleman, Serum estradiol does not differentiate stress, mixed and urge incontinent women around menopause. A report from the Women's Health in the Lund Area (WHILA) study. Eur J Obstet Gynecol Reprod Biol 2011;159:209-12.
[173] Smith, P., Estrogens and the urogenital tract. Studies on steroid hormone receptors and a clinical study on a new estradiol-releasing vaginal ring. Acta Obstet Gynecol Scand Suppl 1993;157:1-26.
[174] Cody, J.D., M.L. Jacobs, K. Richardson, B. Moehrer, and A. Hextall, Oestrogen therapy for urinary incontinence in post-menopausal women. Cochrane Database Syst Rev 2012;10:CD001405.
[175] Clinical Applications of Biomaterials. National Institutes of Health Consensus Development Conference Statement in NIH Consensus Statement Online 1982, National Institutes of Health. p. 1-19..
[176] Matsuoka, P.K., R.F. Locali, A.M. Pacetta, E.C. Baracat, and J.M. Haddad, The

efficacy and safety of urethral injection therapy for urinary incontinence in women: a systematic review. Clinics (Sao Paulo) 2016;71:94-100.
[177] Lee, P.E., R.C. Kung, and H.P. Drutz, Periurethral autologous fat injection as treatment for female stress urinary incontinence: a randomized double-blind controlled trial. J Urol 2001;165:153-8.
[178] Kuhn, A., W. Stadlmayr, D. Lengsfeld, and M.D. Mueller, Where should bulking agents for female urodynamic stress incontinence be injected? Int Urogynecol J Pelvic Floor Dysfunct 2008;19:817-21.
[179] Ghoniem, G., J. Corcos, C. Comiter, P. Bernhard, O.L. Westney, and S. Herschorn, Cross-linked polydimethylsiloxane injection for female stress urinary incontinence: results of a multicenter, randomized, controlled, single-blind study. J Urol 2009;181:204-10.
[180] Bano, F., J.W. Barrington, and R. Dyer, Comparison between porcine dermal implant (Permacol) and silicone injection (Macroplastique) for urodynamic stress incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2005;16:147-50; discussion 150.
[181] Andersen, R.C., Long-term follow-up comparison of durasphere and contigen in the treatment of stress urinary incontinence. J Low Genit Tract Dis 2002;6:239-43.
[182] Mayer, R.D., R.R. Dmochowski, R.A. Appell, P.K. Sand, I.W. Klimberg, K. Jacoby, C.W. Graham, J.A. Snyder, V.W. Nitti, and J.C. Winters, Multicenter prospective randomized 52-week trial of calcium hydroxylapatite versus bovine dermal collagen for treatment of stress urinary incontinence. Urology 2007;69:876-80.
[183] Lightner, D., C. Calvosa, R. Andersen, I. Klimberg, C.G. Brito, J. Snyder, D. Gleason, D. Killion, J. Macdonald, A.U. Khan, A. Diokno, L.T. Sirls, and D. Saltzstein, A new injectable bulking agent for treatment of stress urinary incontinence: results of a multicenter, randomized, controlled, double-blind study of Durasphere. Urology 2001;58:12-5.
[184] Lightner, D., E. Rovner, J. Corcos, C. Payne, L. Brubaker, H. Drutz, G. Steinhoff, and G. Zuidex Study, Randomized controlled multisite trial of injected bulking agents for women with intrinsic sphincter deficiency: mid-urethral injection of Zuidex via the Implacer versus proximal urethral injection of Contigen cystoscopically. Urology 2009;74:771-5.
[185] Sokol, E.R., M.M. Karram, and R. Dmochowski, Efficacy and safety of polyacrylamide hydrogel for the treatment of female stress incontinence: a randomized, prospective, multicenter North American study. J Urol 2014;192:843-9.
[186] Amid, P.K., Classification of biomaterials and their relative complications in an abdominal wall hernia surgery. . Hernia 1997;1:15–21..
[187] Moalli, P.A., Cadaveric fascia lata. Int Urogynecol J Pelvic Floor Dysfunct 2006;17 Suppl 1:S48-50.
[188] Lemer, M.L., D.C. Chaikin, and J.G. Blaivas, Tissue strength analysis of autologous and cadaveric allografts for the pubovaginal sling. Neurourol Urodyn 1999;18:497-503.
[189] Choe, J.M. and T. Bell, Genetic material is present in cadaveric dermis and cadaveric fascia lata. J Urol 2001;166:122-4.
[190] Zheng, M.H., J. Chen, Y. Kirilak, C. Willers, J. Xu, and D. Wood, Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains

porcine DNA: possible implications in human implantation. J Biomed Mater Res B Appl Biomater 2005;73:61-7.
[191] Marshall, V.F., A.A. Marchetti, and K.E. Krantz, The correction of stress incontinence by simple vesicourethral suspension. Surg Gynecol Obstet 1949;88:509-18.
[192] Mainprize, T.C. and H.P. Drutz, The Marshall-Marchetti-Krantz procedure: a critical review. Obstet Gynecol Surv 1988;43:724-9.
[193] Jarvis, G.J., Surgery for genuine stress incontinence. Br J Obstet Gynaecol 1994;101:371-4.
[194] Lapitan, M.C. and J.D. Cody, Open retropubic colposuspension for urinary incontinence in women. Cochrane Database Syst Rev 2012;6:CD002912.
[195] Maher, C.F., P.L. Dwyer, M.P. Carey, and P.A. Moran, Colposuspension or sling for low urethral pressure stress incontinence? Int Urogynecol J Pelvic Floor Dysfunct 1999;10:384-9.
[196] White, G.R., Cystocele--a radical cure by suturing lateral sulci of the vagina to the white line of pelvic fascia. 1909. Int Urogynecol J Pelvic Floor Dysfunct 1997;8:288-92.
[197] German, K.A., H. Kynaston, S. Weight, and T.P. Stephenson, A prospective randomized trial comparing a modified needle suspension procedure with the vagina/obturator shelf procedure for genuine stress incontinence. Br J Urol 1994;74:188-90.
[198] Carey, M.P., J.T. Goh, A. Rosamilia, A. Cornish, I. Gordon, G. Hawthorne, C.F. Maher, P.L. Dwyer, P. Moran, and D.T. Gilmour, Laparoscopic versus open Burch colposuspension: a randomised controlled trial. BJOG 2006;113:999-1006.
[199] Kitchener, H.C., G. Dunn, V. Lawton, F. Reid, L. Nelson, A.R. Smith, and C.S. Group, Laparoscopic versus open colposuspension--results of a prospective randomised controlled trial. BJOG 2006;113:1007-13.
[200] Woodruff, A.J., E.E. Cole, R.R. Dmochowski, H.M. Scarpero, E.N. Beckman, and J.C. Winters, Histologic comparison of pubovaginal sling graft materials: a comparative study. Urology 2008;72:85-9.
[201] McBride, A.W., R.M. Ellerkmann, A.E. Bent, and C.F. Melick, Comparison of long-term outcomes of autologous fascia lata slings with Suspend Tutoplast fascia lata allograft slings for stress incontinence. Am J Obstet Gynecol 2005;192:1677-81.
[202] Ulmsten, U. and P. Petros, Intravaginal slingplasty (IVS): an ambulatory surgical procedure for treatment of female urinary incontinence. Scand J Urol Nephrol 1995;29:75-82.
[203] Delorme, E., [Transobturator urethral suspension: mini-invasive procedure in the treatment of stress urinary incontinence in women]. Prog Urol 2001;11:1306-13.
[204] de Leval, J., Novel surgical technique for the treatment of female stress urinary incontinence: transobturator vaginal tape inside-out. Eur Urol 2003;44:724-30.
[205] Corcos, J., J.P. Collet, S. Shapiro, S. Herschorn, S.B. Radomski, E. Schick, J.B. Gajewski, A. Benedetti, E. MacRamallah, and B. Hyams, Multicenter randomized clinical trial comparing surgery and collagen injections for treatment of female stress urinary incontinence. Urology 2005;65:898-904.
[206] Maher, C.F., B.A. O'Reilly, P.L. Dwyer, M.P. Carey, A. Cornish, and P. Schluter,

Pubovaginal sling versus transurethral Macroplastique for stress urinary incontinence and intrinsic sphincter deficiency: a prospective randomised controlled trial. BJOG 2005;112:797-801.
[207] Gaddi, A., N. Guaderrama, N. Bassiouni, J. Bebchuk, and E.L. Whitcomb, Repeat midurethral sling compared with urethral bulking for recurrent stress urinary incontinence. Obstet Gynecol 2014;123:1207-12.
[208] Albo, M.E., H.E. Richter, L. Brubaker, P. Norton, S.R. Kraus, P.E. Zimmern, T.C. Chai, H. Zyczynski, A.C. Diokno, S. Tennstedt, C. Nager, L.K. Lloyd, M. FitzGerald, G.E. Lemack, H.W. Johnson, W. Leng, V. Mallett, A.M. Stoddard, S. Menefee, R.E. Varner, K. Kenton, P. Moalli, L. Sirls, K.J. Dandreo, J.W. Kusek, L.M. Nyberg, and W. Steers, Burch colposuspension versus fascial sling to reduce urinary stress incontinence. N Engl J Med 2007;356:2143-55.
[209] Demirci, F. and O. Yucel, Comparison of pubovaginal sling and burch colposuspension procedures in type I/II genuine stress incontinence. Arch Gynecol Obstet 2001;265:190-4.
[210] Bai, S.W., W.H. Sohn, D.J. Chung, J.H. Park, and S.K. Kim, Comparison of the efficacy of Burch colposuspension, pubovaginal sling, and tension-free vaginal tape for stress urinary incontinence. Int J Gynaecol Obstet 2005;91:246-51.
[211] Ogah, J., J.D. Cody, and L. Rogerson, Minimally invasive synthetic suburethral sling operations for stress urinary incontinence in women. Cochrane Database Syst Rev 2009;CD006375.
[212] Kondo, A., Y. Isobe, K. Kimura, O. Kamihira, O. Matsuura, M. Gotoh, and H. Ozawa, Efficacy, safety and hospital costs of tension-free vaginal tape and pubovaginal sling in the surgical treatment of stress incontinence. J Obstet Gynaecol Res 2006;32:539-44.
[213] Sharifiaghdas, F. and N. Mortazavi, Tension-free vaginal tape and autologous rectus fascia pubovaginal sling for the treatment of urinary stress incontinence: a medium-term follow-up. Med Princ Pract 2008;17:209-14.
[214] Wadie, B.S., A. Edwan, and A.M. Nabeeh, Autologous fascial sling vs polypropylene tape at short-term followup: a prospective randomized study. J Urol 2005;174:990-3.
[215] Basok, E.K., A. Yildirim, N. Atsu, A. Basaran, and R. Tokuc, Cadaveric fascia lata versus intravaginal slingplasty for the pubovaginal sling: surgical outcome, overall success and patient satisfaction rates. Urol Int 2008;80:46-51.
[216] Paparella, R., M. Marturano, L. Pelino, A. Scarpa, G. Scambia, G. La Torre, and P. Paparella, Prospective randomized trial comparing synthetic vs biological out-in transobturator tape: a mean 3-year follow-up study. Int Urogynecol J 2010;21:1327-36.
[217] Ugurlucan, F.G., H.A. Erkan, M. Onal, and O. Yalcin, Randomized trial of graft materials in transobturator tape operation: biological versus synthetic. Int Urogynecol J 2013;24:1315-23.
[218] DeLancey, J.O., The anatomy of the pelvic floor. Curr Opin Obstet Gynecol 1994;6:313-6.
[219] Jha, S. and P. Moran, The UK national prolapse survey: 5 years on. Int Urogynecol J 2011;22:517-28.
[220] Barber, M.D. and C. Maher, Apical prolapse. Int Urogynecol J 2013;24:1815-33.
[221] Margulies, R.U., M.A. Rogers, and D.M. Morgan, Outcomes of transvaginal uterosacral ligament suspension: systematic review and metaanalysis. Am J Obstet Gynecol 2010;202:124-34.

[222] Morgan, D.M., M.A. Rogers, M. Huebner, J.T. Wei, and J.O. Delancey, Heterogeneity in anatomic outcome of sacrospinous ligament fixation for prolapse: a systematic review. Obstet Gynecol 2007;109:1424-33.
[223] Barber, M.D., L. Brubaker, K.L. Burgio, H.E. Richter, I. Nygaard, A.C. Weidner, S.A. Menefee, E.S. Lukacz, P. Norton, J. Schaffer, J.N. Nguyen, D. Borello-France, P.S. Goode, S. Jakus-Waldman, C. Spino, L.K. Warren, M.G. Gantz, and S.F. Meikle, Comparison of 2 transvaginal surgical approaches and perioperative behavioral therapy for apical vaginal prolapse: the OPTIMAL randomized trial. JAMA 2014;311:1023-34.
[224] Maher, C.F., B. Feiner, E.M. DeCuyper, C.J. Nichlos, K.V. Hickey, and P. O'Rourke, Laparoscopic sacral colpopexy versus total vaginal mesh for vaginal vault prolapse: a randomized trial. Am J Obstet Gynecol 2011;204:360 e1-7.
[225] Maher, C., B. Feiner, K. Baessler, and C. Schmid, Surgical management of pelvic organ prolapse in women. Cochrane Database Syst Rev 2013;4:CD004014.
[226] Nygaard, I.E., R. McCreery, L. Brubaker, A. Connolly, G. Cundiff, A.M. Weber, and H. Zyczynski, Abdominal sacrocolpopexy: a comprehensive review. Obstet Gynecol 2004;104:805-23.
[227] Culligan, P.J., L. Blackwell, L.J. Goldsmith, C.A. Graham, A. Rogers, and M.H. Heit, A randomized controlled trial comparing fascia lata and synthetic mesh for sacral colpopexy. Obstet Gynecol 2005;106:29-37.
[228] Tate, S.B., L. Blackwell, D.J. Lorenz, M.M. Steptoe, and P.J. Culligan, Randomized trial of fascia lata and polypropylene mesh for abdominal sacrocolpopexy: 5-year follow-up. Int Urogynecol J 2011;22:137-43.
[229] Maher, C., Anterior vaginal compartment surgery. Int Urogynecol J 2013;24:1791-802.
[230] Sand, P.K., S. Koduri, R.W. Lobel, H.A. Winkler, J. Tomezsko, P.J. Culligan, and R. Goldberg, Prospective randomized trial of polyglactin 910 mesh to prevent recurrence of cystoceles and rectoceles. Am J Obstet Gynecol 2001;184:1357-62; discussion 1362-4.
[231] Weber, A.M., M.D. Walters, M.R. Piedmonte, and L.A. Ballard, Anterior colporrhaphy: a randomized trial of three surgical techniques. Am J Obstet Gynecol 2001;185:1299-304; discussion 1304-6.
[232] Menefee, S.A., K.Y. Dyer, E.S. Lukacz, A.J. Simsiman, K.M. Luber, and J.N. Nguyen, Colporrhaphy compared with mesh or graft-reinforced vaginal paravaginal repair for anterior vaginal wall prolapse: a randomized controlled trial. Obstet Gynecol 2011;118:1337-44.
[233] Hviid, U., T.V. Hviid, and M. Rudnicki, Porcine skin collagen implants for anterior vaginal wall prolapse: a randomised prospective controlled study. Int Urogynecol J 2010;21:529-34.
[234] Meschia, M., P. Pifarotti, F. Bernasconi, F. Magatti, D. Riva, and E. Kocjancic, Porcine skin collagen implants to prevent anterior vaginal wall prolapse recurrence: a multicenter, randomized study. J Urol 2007;177:192-5.
[235] Ross, J.W., Porcine dermal hammock for repair of anterior and posterior vaginal wall prolapse: 5-year outcome. J Minim Invasive Gynecol 2008;15:459-65.
[236] Natale, F., C. La Penna, A. Padoa, M. Agostini, E. De Simone, and M. Cervigni, A prospective, randomized, controlled study comparing Gynemesh, a synthetic mesh, and Pelvicol, a biologic graft, in the surgical treatment of

recurrent cystocele. Int Urogynecol J Pelvic Floor Dysfunct 2009;20:75-81.
[237] Feldner, P.C., Jr., R.A. Castro, L.A. Cipolotti, C.A. Delroy, M.G. Sartori, and M.J. Girao, Anterior vaginal wall prolapse: a randomized controlled trial of SIS graft versus traditional colporrhaphy. Int Urogynecol J 2010;21:1057-63.
[238] Gandhi, S., R.P. Goldberg, C. Kwon, S. Koduri, J.L. Beaumont, Y. Abramov, and P.K. Sand, A prospective randomized trial using solvent dehydrated fascialata for the prevention of recurrent anterior vaginal wall prolapse. Am J Obstet Gynecol 2005;192:1649-54.
[239] Guerette, N.L., T.V. Peterson, O.A. Aguirre, D.M. Vandrie, D.H. Biller, and G.W. Davila, Anterior repair with or without collagen matrix reinforcement: a randomized controlled trial. Obstet Gynecol 2009;114:59-65.
[240] Karram, M. and C. Maher, Surgery for posterior vaginal wall prolapse. Int Urogynecol J 2013;24:1835-41.
[241] Su, K.C., M.F. Mutone, C.L. Terry, and D.S. Hale, Abdominovaginal sacral colpoperineopexy: patient perceptions, anatomical outcomes, and graft erosions. Int Urogynecol J Pelvic Floor Dysfunct 2007;18:503-11.
[242] Sung, V.W., C.R. Rardin, C.A. Raker, C.A. Lasala, and D.L. Myers, Porcine subintestinal submucosal graft augmentation for rectocele repair: a randomized controlled trial. Obstet Gynecol 2012;119:125-33.
[243] Paraiso, M.F., M.D. Barber, T.W. Muir, and M.D. Walters, Rectocele repair: a randomized trial of three surgical techniques including graft augmentation. Am J Obstet Gynecol 2006;195:1762-71.
[244] Dahlgren, E. and P. Kjolhede, Long-term outcome of porcine skin graft in surgical treatment of recurrent pelvic organ prolapse. An open randomized controlled multicenter study. Acta Obstet Gynecol Scand 2011;90:1393-401.
[245] Walter, A.J., J.G. Hentz, J.F. Magrina, and J.L. Cornella, Harvesting autologous fascia lata for pelvic reconstructive surgery: techniques and morbidity. Am J Obstet Gynecol 2001;185:1354-8; discussion 1459.
[246] FDA public health notification: serious complications associated with transvaginal placement of surgical mesh in repair of pelvic organ prolapse and stress urinary incontinence. . [Issued October 20, 2008]; Available from: http://www.fda.gov/medicaldevices/safety/alertsandnotices/publichealthnotifications/ucm061976.htm..
[247] FDA Safety Communication: UPDATE on Serious Complications Associated with Transvaginal Placement of Surgical Mesh for Pelvic Organ Prolapse. 2011; Available from: http://www.fda.gov/medicaldevices/safety/alertsandnotices/ucm262435.htm..
[248] Haylen, B.T., R.M. Freeman, S.E. Swift, M. Cosson, G.W. Davila, J. Deprest, P.L. Dwyer, B. Fatton, E. Kocjancic, J. Lee, C. Maher, E. Petri, D.E. Rizk, P.K. Sand, G.N. Schaer, R. Webb, A. International Urogynecological, S. International Continence, and I.I.C.S.W.G.o.C.T. Joint, An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint terminology and classification of the complications related directly to the insertion of prostheses (meshes, implants, tapes) and grafts in female pelvic floor surgery. Neurourol Urodyn 2011;30:2-12.
[249] Lee, J.K., P.L. Dwyer, A. Rosamilia, Y.N. Lim, A. Polyakov, and K. Stav, Which women develop urgency or urgency urinary incontinence following midurethral slings? Int Urogynecol J 2013;24:47-54.
[250] Cholhan, H.J., T.B. Hutchings, and K.E. Rooney, Dyspareunia associated with paraurethral banding in the transobturator sling. Am J Obstet Gynecol

2010;202:481 e1-5.
[251] Milani, R., S. Salvatore, M. Soligo, P. Pifarotti, M. Meschia, and M. Cortese, Functional and anatomical outcome of anterior and posterior vaginal prolapse repair with prolene mesh. BJOG 2005;112:107-11.
[252] Abed, H., D.D. Rahn, L. Lowenstein, E.M. Balk, J.L. Clemons, R.G. Rogers, and S. Systematic Review Group of the Society of Gynecologic, Incidence and management of graft erosion, wound granulation, and dyspareunia following vaginal prolapse repair with graft materials: a systematic review. Int Urogynecol J 2011;22:789-98.
[253] Brubaker, L., P.A. Norton, M.E. Albo, T.C. Chai, K.J. Dandreo, K.L. Lloyd, J.L. Lowder, L.T. Sirls, G.E. Lemack, A.M. Arisco, Y. Xu, J.W. Kusek, and N. Urinary Incontinence Treatment, Adverse events over two years after retropubic or transobturator midurethral sling surgery: findings from the Trial of Midurethral Slings (TOMUS) study. Am J Obstet Gynecol 2011;205:498 e1-6.
[254] Khanuengkitkong, S., T.S. Lo, and A.K. Dass, Delayed vaginal and urethral mesh exposure: 10 years after TVT surgery. Int Urogynecol J 2013;24:519-21.
[255] Smith, A.R., W. Artibani, and M.J. Drake, Managing unsatisfactory outcome after mid-urethral tape insertion. Neurourol Urodyn 2011;30:771-4.
[256] Anderson, J.M., A. Rodriguez, and D.T. Chang, Foreign body reaction to biomaterials. Semin Immunol 2008;20:86-100.
[257] Klosterhalfen, B., U. Klinge, B. Hermanns, and V. Schumpelick, [Pathology of traditional surgical nets for hernia repair after long-term implantation in humans]. Chirurg 2000;71:43-51.
[258] Pierce, L.M., J.R. Asarias, P.T. Nguyen, J.R. Mings, and A.P. Gehrich, Inflammatory cytokine and matrix metalloproteinase expression induced by collagen-coated and uncoated polypropylene meshes in a rat model. Am J Obstet Gynecol 2011;205:82 e1-9.
[259] Rechberger, T., K. Jankiewicz, A. Adamiak, P. Miotla, A. Chrobak, and M. Jerzak, Do preoperative cytokine levels offer a prognostic factor for polypropylene mesh erosion after suburethral sling surgery for stress urinary incontinence? Int Urogynecol J Pelvic Floor Dysfunct 2009;20:69-74.
[260] Peacock, E. and W. Van Winkle, Structure, synthesis and interaction of fibrous protein and matrix. Wound Repair 1984;56-101.
[261] Birch, C. and M.M. Fynes, The role of synthetic and biological prostheses in reconstructive pelvic floor surgery. Curr Opin Obstet Gynecol 2002;14:527-35.
[262] Deprest, J., F. Zheng, M. Konstantinovic, F. Spelzini, F. Claerhout, A. Steensma, Y. Ozog, and D. De Ridder, The biology behind fascial defects and the use of implants in pelvic organ prolapse repair. Int Urogynecol J Pelvic Floor Dysfunct 2006;17 Suppl 1:S16-25.
[263] Li, X., J.A. Kruger, J.W. Jor, V. Wong, H.P. Dietz, M.P. Nash, and P.M. Nielsen, Characterizing the ex vivo mechanical properties of synthetic polypropylene surgical mesh. J Mech Behav Biomed Mater 2014;37:48-55.
[264] Manodoro, S., M. Endo, P. Uvin, M. Albersen, J. Vlacil, A. Engels, B. Schmidt, D. De Ridder, A. Feola, and J. Deprest, Graft-related complications and biaxial tensiometry following experimental vaginal implantation of flat mesh of variable dimensions. BJOG 2013;120:244-50.
[265] Kruger, J.A., P.M. Nielsen, S.C. Budgett, and A.J. Taberner, An automated hand-held elastometer for quantifying the passive stiffness of the levator ani muscle in women. Neurourol Urodyn 2013;.

[266] Clave, A., H. Yahi, J.C. Hammou, S. Montanari, P. Gounon, and H. Clave, Polypropylene as a reinforcement in pelvic surgery is not inert: comparative analysis of 100 explants. Int Urogynecol J 2010;21:261-70.
[267] Frostling, H., A. Hoff, S. Jacobsson, P. Pfaffli, S. Vainiotalo, and A. Zitting, Analytical, occupational and toxicologic aspects of the degradation products of polypropylene plastics. Scand J Work Environ Health 1984;10:163-9.
[268] Blanchard, K.T., C. Barthel, J.E. French, H.E. Holden, R. Moretz, F.D. Pack, R.W. Tennant, and R.E. Stoll, Transponder-induced sarcoma in the heterozygous p53+/- mouse. Toxicol Pathol 1999;27:519-27.
[269] Vollmar, J. and G. Ott, [Experimental tumor induction by plastics from a surgical point of view]. Langenbecks Arch Klin Chir Ver Dtsch Z Chir 1961;298:729-35.
[270] Witherspoon, P., G. Bryson, D.M. Wright, R. Reid, and P.J. O'Dwyer, Carcinogenic potential of commonly used hernia repair prostheses in an experimental model. Br J Surg 2004;91:368-72.
[271] Falagas, M.E., S. Velakoulis, C. Iavazzo, and S. Athanasiou, Mesh-related infections after pelvic organ prolapse repair surgery. Eur J Obstet Gynecol Reprod Biol 2007;134:147-56.
[272] Bako, A. and R. Dhar, Review of synthetic mesh-related complications in pelvic floor reconstructive surgery. Int Urogynecol J Pelvic Floor Dysfunct 2009;20:103-11.
[273] Shah, H.N. and G.H. Badlani, Mesh complications in female pelvic floor reconstructive surgery and their management: A systematic review. Indian J Urol 2012;28:129-53.
[274] Chen, X., X. Tong, M. Jiang, H. Li, J. Qiu, L. Shao, and X. Yang, A modified inexpensive transobturator vaginal tape inside-out procedure versus tension-free vaginal tape for the treatment of SUI: a prospective comparative study. Arch Gynecol Obstet 2011;284:1461-6.
[275] Murray, S., R.M. Haverkorn, Y. Lotan, and G.E. Lemack, Mesh kits for anterior vaginal prolapse are not cost effective. Int Urogynecol J 2011;22:447-52.
[276] Baessler, K., A.D. Hewson, R. Tunn, B. Schuessler, and C.F. Maher, Severe mesh complications following intravaginal slingplasty. Obstet Gynecol 2005;106:713-6.
[277] Wei, J.T., I. Nygaard, H.E. Richter, C.W. Nager, M.D. Barber, K. Kenton, C.L. Amundsen, J. Schaffer, S.F. Meikle, C. Spino, and N. Pelvic Floor Disorders, A midurethral sling to reduce incontinence after vaginal prolapse repair. N Engl J Med 2012;366:2358-67.
[278] Carr, L.K. and G.D. Webster, Voiding dysfunction following incontinence surgery: diagnosis and treatment with retropubic or vaginal urethrolysis. J Urol 1997;157:821-3.
[279] Nitti, V.W., K.V. Carlson, J.G. Blaivas, and R.R. Dmochowski, Early results of pubovaginal sling lysis by midline sling incision. Urology 2002;59:47-51; discussion 51-2.
[280] Zubke, W., S. Becker, B. Kramer, and D. Wallwiener, Persistent urinary retention after tension-free vaginal tape: a new surgical solution. Eur J Obstet Gynecol Reprod Biol 2004;115:95-8.
[281] Gomelsky, A., V.W. Nitti, and R.R. Dmochowski, Management of obstructive voiding dysfunction after incontinence surgery: lessons learned. Urology 2003;62:391-9.

[282] Bump, R.C., J.A. Fantl, and W.G. Hurt, Dynamic urethral pressure profilometry pressure transmission ratio determinations after continence surgery: understanding the mechanism of success, failure, and complications. Obstet Gynecol 1988;72:870-4.
[283] Badylak, S.F., The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 2002;13:377-83.
[284] Brennan, E.P., J. Reing, D. Chew, J.M. Myers-Irvin, E.J. Young, and S.F. Badylak, Antibacterial activity within degradation products of biological scaffolds composed of extracellular matrix. Tissue Eng 2006;12:2949-55.
[285] FitzGerald, M.P., J. Mollenhauer, P. Bitterman, and L. Brubaker, Functional failure of fascia lata allografts. Am J Obstet Gynecol 1999;181:1339-44; discussion 1344-6.
[286] Valentin, J.E., A.M. Stewart-Akers, T.W. Gilbert, and S.F. Badylak, Macrophage participation in the degradation and remodeling of extracellular matrix scaffolds. Tissue Eng Part A 2009;15:1687-94.
[287] Cole, E., A. Gomelsky, and R.R. Dmochowski, Encapsulation of a porcine dermis pubovaginal sling. J Urol 2003;170:1950.
[288] Boruch, A.V., A. Nieponice, I.R. Qureshi, T.W. Gilbert, and S.F. Badylak, Constructive remodeling of biologic scaffolds is dependent on early exposure to physiologic bladder filling in a canine partial cystectomy model. J Surg Res 2010;161:217-25.
[289] FitzGerald, M.P., J. Mollenhauer, and L. Brubaker, The fate of rectus fascia suburethral slings. Am J Obstet Gynecol 2000;183:964-6.
[290] Brown, B.N., B.D. Ratner, S.B. Goodman, S. Amar, and S.F. Badylak, Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine. Biomaterials 2012;33:3792-802.
[291] Badylak, S.F., J.E. Valentin, A.K. Ravindra, G.P. McCabe, and A.M. Stewart-Akers, Macrophage phenotype as a determinant of biologic scaffold remodeling. Tissue Eng Part A 2008;14:1835-42.
[292] Karlovsky, M.E., L. Kushner, and G.H. Badlani, Synthetic biomaterials for pelvic floor reconstruction. Curr Urol Rep 2005;6:376-84.
[293] Mantovani, A., A. Sica, and M. Locati, Macrophage polarization comes of age. Immunity 2005;23:344-6.
[294] Claerhout, F., G. Verbist, E. Verbeken, M. Konstantinovic, D. De Ridder, and J. Deprest, Fate of collagen-based implants used in pelvic floor surgery: a 2-year follow-up study in a rabbit model. Am J Obstet Gynecol 2008;198:94 e1-6.
[295] Daar, A.S. and H.L. Greenwood, A proposed definition of regenerative medicine. J Tissue Eng Regen Med 2007;1:179-84.
[296] Langer, R. and J.P. Vacanti, Tissue engineering. Science 1993;260:920-6.
[297] Martinez-Climent, J.A., E.J. Andreu, and F. Prosper, Somatic stem cells and the origin of cancer. Clin Transl Oncol 2006;8:647-63.
[298] Drost, A.C., S. Weng, G. Feil, J. Schafer, S. Baumann, L. Kanz, K.D. Sievert, A. Stenzl, and R. Mohle, In vitro myogenic differentiation of human bone marrow-derived mesenchymal stem cells as a potential treatment for urethral sphincter muscle repair. Ann N Y Acad Sci 2009;1176:135-43.
[299] Kinebuchi, Y., N. Aizawa, T. Imamura, O. Ishizuka, Y. Igawa, and O. Nishizawa, Autologous bone-marrow-derived mesenchymal stem cell transplantation into injured rat urethral sphincter. Int J Urol 2010;17:359-68.
[300] Corcos, J., O. Loutochin, L. Campeau, N. Eliopoulos, M. Bouchentouf, B. Blok,

and J. Galipeau, Bone marrow mesenchymal stromal cell therapy for external urethral sphincter restoration in a rat model of stress urinary incontinence. Neurourol Urodyn 2011;30:447-55.
[301] Pittenger, M.F., A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, and D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
[302] Oshima, H., T.R. Payne, K.L. Urish, T. Sakai, Y. Ling, B. Gharaibeh, K. Tobita, B.B. Keller, J.H. Cummins, and J. Huard, Differential myocardial infarct repair with muscle stem cells compared to myoblasts. Mol Ther 2005;12:1130-41.
[303] Lee, J.Y., Z. Qu-Petersen, B. Cao, S. Kimura, R. Jankowski, J. Cummins, A. Usas, C. Gates, P. Robbins, A. Wernig, and J. Huard, Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. J Cell Biol 2000;150:1085-100.
[304] Qu-Petersen, Z., B. Deasy, R. Jankowski, M. Ikezawa, J. Cummins, R. Pruchnic, J. Mytinger, B. Cao, C. Gates, A. Wernig, and J. Huard, Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. J Cell Biol 2002;157:851-64.
[305] Cannon, T.W., J.Y. Lee, G. Somogyi, R. Pruchnic, C.P. Smith, J. Huard, and M.B. Chancellor, Improved sphincter contractility after allogenic muscle-derived progenitor cell injection into the denervated rat urethra. Urology 2003;62:958-63.
[306] Chancellor, M.B., T. Yokoyama, S. Tirney, C.E. Mattes, H. Ozawa, N. Yoshimura, W.C. de Groat, and J. Huard, Preliminary results of myoblast injection into the urethra and bladder wall: a possible method for the treatment of stress urinary incontinence and impaired detrusor contractility. Neurourol Urodyn 2000;19:279-87.
[307] Chermansky, C.J., T. Tarin, D.D. Kwon, R.J. Jankowski, T.W. Cannon, W.C. de Groat, J. Huard, and M.B. Chancellor, Intraurethral muscle-derived cell injections increase leak point pressure in a rat model of intrinsic sphincter deficiency. Urology 2004;63:780-5.
[308] Lee, J.Y., T.W. Cannon, R. Pruchnic, M.O. Fraser, J. Huard, and M.B. Chancellor, The effects of periurethral muscle-derived stem cell injection on leak point pressure in a rat model of stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2003;14:31-7; discussion 37.
[309] Lee, J.Y., S.Y. Paik, S.H. Yuk, J.H. Lee, S.H. Ghil, and S.S. Lee, Long term effects of muscle-derived stem cells on leak point pressure and closing pressure in rats with transected pudendal nerves. Mol Cells 2004;18:309-13.
[310] Tamaki, T., Y. Uchiyama, Y. Okada, T. Ishikawa, M. Sato, A. Akatsuka, and T. Asahara, Functional recovery of damaged skeletal muscle through synchronized vasculogenesis, myogenesis, and neurogenesis by muscle-derived stem cells. Circulation 2005;112:2857-66.
[311] Mitterberger, M., R. Marksteiner, E. Margreiter, G.M. Pinggera, D. Colleselli, F. Frauscher, H. Ulmer, M. Fussenegger, G. Bartsch, and H. Strasser, Autologous myoblasts and fibroblasts for female stress incontinence: a 1-year follow-up in 123 patients. BJU Int 2007;100:1081-5.
[312] Carr, L.K., D. Steele, S. Steele, D. Wagner, R. Pruchnic, R. Jankowski, J. Erickson, J. Huard, and M.B. Chancellor, 1-year follow-up of autologous muscle-derived stem cell injection pilot study to treat stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:881-3.

[313] Gras, S., N. Klarskov, and G. Lose, Intraurethral injection of autologous minced skeletal muscle: a simple surgical treatment for stress urinary incontinence. J Urol 2014;192:850-5.
[314] Roche, R., F. Festy, and X. Fritel, Stem cells for stress urinary incontinence: the adipose promise. J Cell Mol Med 2010;14:135-42.
[315] Ning, H., G. Lin, T.F. Lue, and C.S. Lin, Neuron-like differentiation of adipose tissue-derived stromal cells and vascular smooth muscle cells. Differentiation 2006;74:510-8.
[316] Zuk, P.A., M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, and M.H. Hedrick, Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211-28.
[317] Miranville, A., C. Heeschen, C. Sengenes, C.A. Curat, R. Busse, and A. Bouloumie, Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 2004;110:349-55.
[318] Miyahara, Y., N. Nagaya, M. Kataoka, B. Yanagawa, K. Tanaka, H. Hao, K. Ishino, H. Ishida, T. Shimizu, K. Kangawa, S. Sano, T. Okano, S. Kitamura, and H. Mori, Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 2006;12:459-65.
[319] Rodriguez, L.V., Z. Alfonso, R. Zhang, J. Leung, B. Wu, and L.J. Ignarro, Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proc Natl Acad Sci U S A 2006;103:12167-72.
[320] Bacou, F., R.B. el Andalousi, P.A. Daussin, J.P. Micallef, J.M. Levin, M. Chammas, L. Casteilla, Y. Reyne, and J. Nougues, Transplantation of adipose tissue-derived stromal cells increases mass and functional capacity of damaged skeletal muscle. Cell Transplant 2004;13:103-11.
[321] Gotoh, M., T. Yamamoto, M. Kato, T. Majima, K. Toriyama, Y. Kamei, Y. Matsukawa, A. Hirakawa, and Y. Funahashi, Regenerative treatment of male stress urinary incontinence by periurethral injection of autologous adipose-derived regenerative cells: 1-year outcomes in 11 patients. Int J Urol 2014;21:294-300.
[322] Kuismanen, K., R. Sartoneva, S. Haimi, B. Mannerstrom, E. Tomas, S. Miettinen, and K. Nieminen, Autologous adipose stem cells in treatment of female stress urinary incontinence: results of a pilot study. Stem Cells Transl Med 2014;3:936-41.
[323] Guerrero, K., A. Watkins, S. Emery, K. Wareham, T. Stephenson, V. Logan, and M. Lucas, A randomised controlled trial comparing two autologous fascial sling techniques for the treatment of stress urinary incontinence in women: short, medium and long-term follow-up. Int Urogynecol J Pelvic Floor Dysfunct 2007;18:1263-70.
[324] Roman, S., A. Mangera, N.I. Osman, A.J. Bullock, C.R. Chapple, and S. Macneil, Developing a tissue engineered repair material for treatment of stress urinary incontinence and pelvic organ prolapse-which cell source? Neurourol Urodyn 2013;.
[325] Jackson, W.M., L.J. Nesti, and R.S. Tuan, Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. Stem Cell Res Ther 2012;3:20.
[326] Zou, X.H., Y.L. Zhi, X. Chen, H.M. Jin, L.L. Wang, Y.Z. Jiang, Z. Yin, and H.W. Ouyang, Mesenchymal stem cell seeded knitted silk sling for the treatment of stress urinary incontinence. Biomaterials 2010;31:4872-9.

[327] Cannon, T.W., D.D. Sweeney, D.A. Conway, I. Kamo, N. Yoshimura, M. Sacks, and M.B. Chancellor, A tissue-engineered suburethral sling in an animal model of stress urinary incontinence. BJU Int 2005;96:664-9.
[328] Mangera, A., A.J. Bullock, S. Roman, C.R. Chapple, and S. MacNeil, Comparison of candidate scaffolds for tissue engineering for stress urinary incontinence and pelvic organ prolapse repair. BJU Int 2013;112:674-85.
[329] Boennelycke, M., L. Christensen, L.F. Nielsen, S. Gras, and G. Lose, Fresh muscle fiber fragments on a scaffold in rats-a new concept in urogynecology? Am J Obstet Gynecol 2011;205:235 e10-4.
[330] Hung, M.J., M.C. Wen, C.N. Hung, E.S. Ho, G.D. Chen, and V.C. Yang, Tissue-engineered fascia from vaginal fibroblasts for patients needing reconstructive pelvic surgery. Int Urogynecol J 2010;21:1085-93.
[331] Bhargava, S., J.M. Patterson, R.D. Inman, S. MacNeil, and C.R. Chapple, Tissue-engineered buccal mucosa urethroplasty-clinical outcomes. Eur Urol 2008;53:1263-9.
[332] Bergmeister, H., N. Seyidova, C. Schreiber, M. Strobl, C. Grasl, I. Walter, B. Messner, S. Baudis, S. Frohlich, M. Marchetti-Deschmann, M. Griesser, M.D. Franco, M. Krssak, R. Liska, and H. Schima, Biodegradable, Thermoplastic Polyurethane Grafts For Small Diameter Vascular Replacement. Acta Biomater 2014;.
[333] Takanari, K., Y. Hong, R. Hashizume, A. Huber, N.J. Amoroso, A. D'Amore, S.F. Badylak, and W.R. Wagner, Abdominal wall reconstruction by a regionally distinct biocomposite of extracellular matrix digest and a biodegradable elastomer. J Tissue Eng Regen Med 2013;.
[334] Hiles, M. and J. Hodde, Tissue engineering a clinically useful extracellular matrix biomaterial. Int Urogynecol J Pelvic Floor Dysfunct 2006;17 Suppl 1:S39-43.
[335] Reing, J.E., B.N. Brown, K.A. Daly, J.M. Freund, T.W. Gilbert, S.X. Hsiong, A. Huber, K.E. Kullas, S. Tottey, M.T. Wolf, and S.F. Badylak, The effects of processing methods upon mechanical and biologic properties of porcine dermal extracellular matrix scaffolds. Biomaterials 2010;31:8626-33.
[336] Hodde, J.P., D.M. Ernst, and M.C. Hiles, An investigation of the long-term bioactivity of endogenous growth factor in OASIS Wound Matrix. J Wound Care 2005;14:23-5.
[337] Lu, S.H., T.W. Cannon, C. Chermanski, R. Pruchnic, G. Somogyi, M. Sacks, W.C. de Groat, J. Huard, and M.B. Chancellor, Muscle-derived stem cells seeded into acellular scaffolds develop calcium-dependent contractile activity that is modulated by nicotinic receptors. Urology 2003;61:1285-91.
[338] Ho, M.H., S. Heydarkhan, D. Vernet, I. Kovanecz, M.G. Ferrini, N.N. Bhatia, and N.F. Gonzalez-Cadavid, Stimulating vaginal repair in rats through skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds. Obstet Gynecol 2009;114:300-9.
[339] Jiang, H., Y. Hu, Y. Li, P. Zhao, K. Zhu, and W. Chen, A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents. J Control Release 2005;108:237-43.
[340] Venugopal, J. and S. Ramakrishna, Applications of polymer nanofibers in biomedicine and biotechnology. Appl Biochem Biotechnol 2005;125:147-58.
[341] Zhang, Y., C.T. Lim, S. Ramakrishna, and Z.M. Huang, Recent development of polymer nanofibers for biomedical and biotechnological applications. J Mater

Sci Mater Med 2005;16:933-46.
[342] Immich, A.P., M.L. Arias, N. Carreras, R.L. Boemo, and J.A. Tornero, Drug delivery systems using sandwich configurations of electrospun poly(lactic acid) nanofiber membranes and ibuprofen. Mater Sci Eng C Mater Biol Appl 2013;33:4002-8.
[343] Kenawy el, R., G.L. Bowlin, K. Mansfield, J. Layman, D.G. Simpson, E.H. Sanders, and G.E. Wnek, Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. J Control Release 2002;81:57-64.
[344] Hong, Y., K. Fujimoto, R. Hashizume, J. Guan, J.J. Stankus, K. Tobita, and W.R. Wagner, Generating elastic, biodegradable polyurethane/poly(lactide-co-glycolide) fibrous sheets with controlled antibiotic release via two-stream electrospinning. Biomacromolecules 2008;9:1200-7.
[345] Thakur, R.A., C.A. Florek, J. Kohn, and B.B. Michniak, Electrospun nanofibrous polymeric scaffold with targeted drug release profiles for potential application as wound dressing. Int J Pharm 2008;364:87-93.
[346] Canton, I., R. McKean, M. Charnley, K.A. Blackwood, C. Fiorica, A.J. Ryan, and S. MacNeil, Development of an Ibuprofen-releasing biodegradable PLA/PGA electrospun scaffold for tissue regeneration. Biotechnol Bioeng 2010;105:396-408.
[347] Ngawhirunpat, T., P. Opanasopit, T. Rojanarata, P. Akkaramongkolporn, U. Ruktanonchai, and P. Supaphol, Development of meloxicam-loaded electrospun polyvinyl alcohol mats as a transdermal therapeutic agent. Pharm Dev Technol 2009;14:70-9.
[348] Nikkola, L., J. Seppala, A. Harlin, A. Ndreu, and N. Ashammakhi, Electrospun multifunctional diclofenac sodium releasing nanoscaffold. J Nanosci Nanotechnol 2006;6:3290-5.
[349] Lu, Y., H. Jiang, K. Tu, and L. Wang, Mild immobilization of diverse macromolecular bioactive agents onto multifunctional fibrous membranes prepared by coaxial electrospinning. Acta Biomater 2009;5:1562-74.
[350] Luong-Van, E., L. Grondahl, K.N. Chua, K.W. Leong, V. Nurcombe, and S.M. Cool, Controlled release of heparin from poly(epsilon-caprolactone) electrospun fibers. Biomaterials 2006;27:2042-50.
[351] Losordo, D.W. and J.M. Isner, Estrogen and angiogenesis: A review. Arterioscler Thromb Vasc Biol 2001;21:6-12.
[352] Mitsos, S., K. Katsanos, E. Koletsis, G.C. Kagadis, N. Anastasiou, A. Diamantopoulos, D. Karnabatidis, and D. Dougenis, Therapeutic angiogenesis for myocardial ischemia revisited: basic biological concepts and focus on latest clinical trials. Angiogenesis 2012;15:1-22.
[353] Rubanyi, G.M., A. Johns, and K. Kauser, Effect of estrogen on endothelial function and angiogenesis. Vascular Pharmacology 2002;38:89-98.
[354] Oh, B. and C.H. Lee, Advanced Cardiovascular Stent Coated with Nanofiber. Molecular Pharmaceutics 2013;10:4432-4442.
[355] Unnithan, A.R., A.R.K. Sasikala, P. Murugesan, M. Gurusamy, D. Wu, C.H. Park, and C.S. Kim, Electrospun polyurethane-dextran nanofiber mats loaded with Estradiol for post-menopausal wound dressing. International Journal of Biological Macromolecules 2015;77:1-8.
[356] van Laarhoven, J.A., M.A. Kruft, and H. Vromans, In vitro release properties of etonogestrel and ethinyl estradiol from a contraceptive vaginal ring. Int J

Pharm 2002;232:163-73.
[357] Oh, B. and C.H. Lee, Advanced cardiovascular stent coated with nanofiber. Mol Pharm 2013;10:4432-42.
[358] Ghosh, A.K., Factors involved in the regulation of type I collagen gene expression: implication in fibrosis. Exp Biol Med (Maywood) 2002;227:301-14.
[359] Boyera, N., I. Galey, and B.A. Bernard, Effect of vitamin C and its derivatives on collagen synthesis and cross-linking by normal human fibroblasts. Int J Cosmet Sci 1998;20:151-8.
[360] Bean, A.C., A.J. Almarza, and K.A. Athanasiou, Effects of ascorbic acid concentration on the tissue engineering of the temporomandibular joint disc. Proc Inst Mech Eng H 2006;220:439-47.
[361] Cimini, M., D.R. Boughner, J.A. Ronald, D.E. Johnston, and K.A. Rogers, Dermal fibroblasts cultured on small intestinal submucosa: Conditions for the formation of a neotissue. J Biomed Mater Res A 2005;75:895-906.
[362] Mangera, A., A.J. Bullock, C.R. Chapple, and S. Macneil, Are biomechanical properties predictive of the success of prostheses used in stress urinary incontinence and pelvic organ prolapse? A systematic review. Neurourol Urodyn 2012;31:13-21.
[363] Roman Regueros, S., M. Albersen, S. Manodoro, S. Zia, N.I. Osman, A.J. Bullock, C.R. Chapple, J. Deprest, and S. MacNeil, Acute in vivo response to an alternative implant for urogynecology. Biomed Res Int 2014;2014:853610.
[364] Zia, S., J. Toelen, M. Mori da Cunha, P. Dekoninck, P. de Coppi, and J. Deprest, Routine clonal expansion of mesenchymal stem cells derived from amniotic fluid for perinatal applications. Prenat Diagn 2013;33:921-8.
[365] Kaewkhaw, R., A.M. Scutt, and J.W. Haycock, Anatomical site influences the differentiation of adipose-derived stem cells for Schwann-cell phenotype and function. Glia 2011;59:734-49.
[366] Hutmacher, D.W., Scaffold design and fabrication technologies for engineering tissues--state of the art and future perspectives. J Biomater Sci Polym Ed 2001;12:107-24.
[367] O'Brien, F., Biomaterials and scaffolds for tissue engineering. Materials today 2011;14:88-95.
[368] Gunatillake, P.A. and R. Adhikari, Biodegradable synthetic polymers for tissue engineering. Eur Cell Mater 2003;5:1-16; discussion 16.
[369] Sill, T.J. and H.A. von Recum, Electrospinning: applications in drug delivery and tissue engineering. Biomaterials 2008;29:1989-2006.
[370] Stokes, K., R. McVenes, and J.M. Anderson, Polyurethane elastomer biostability. J Biomater Appl 1995;9:321-54.
[371] Szycher, M., A.A. Siciliano, and A.M. Reed, Polyurethanes in medical devices. Med Des Mater 1991;1:18-25.
[372] Guan, J., K.L. Fujimoto, M.S. Sacks, and W.R. Wagner, Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. Biomaterials 2005;26:3961-71.
[373] Milleret, V., B. Simona, P. Neuenschwander, and H. Hall, Tuning electrospinning parameters for production of 3D-fiber-fleeces with increased porosity for soft tissue engineering applications. Eur Cell Mater 2011;21:286-303.
[374] Soliman, S., S. Pagliari, A. Rinaldi, G. Forte, R. Fiaccavento, F. Pagliari, O. Franzese, M. Minieri, P. Di Nardo, S. Licoccia, and E. Traversa, Multiscale

three-dimensional scaffolds for soft tissue engineering via multimodal electrospinning. Acta Biomater 2010;6:1227-37.
[375] Chen, M., P.K. Patra, S.B. Warner, and S. Bhowmick, Role of fiber diameter in adhesion and proliferation of NIH 3T3 fibroblast on electrospun polycaprolactone scaffolds. Tissue Eng 2007;13:579-87.
[376] Yang, S., K.F. Leong, Z. Du, and C.K. Chua, The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng 2001;7:679-89.
[377] Sirivisoot, S. and B.S. Harrison, Skeletal myotube formation enhanced by electrospun polyurethane carbon nanotube scaffolds. Int J Nanomedicine 2011;6:2483-97.
[378] Roman, S., I. Urbankova, G. Callewaert, F. Lesage, C. Hillary, N.I. Osman, C.R. Chapple, J. Deprest, and S. MacNeil, Evaluating Alternative Materials for the Treatment of Stress Urinary Incontinence and Pelvic Organ Prolapse: A Comparison of the In Vivo Response to Meshes Implanted in Rabbits. J Urol 2016;196:261-9.
[379] Petros, P. and J. Papadimitriou, Evolution of midurethral and other mesh slings--a critical analysis. Neurourol Urodyn 2013;32:399-406.
[380] Papadimitriou, J. and P. Petros, Histological studies of monofilament and multifilament polypropylene mesh implants demonstrate equivalent penetration of macrophages between fibrils. Hernia 2005;9:75-8.
[381] Gigliobianco, G., S.R. Regueros, N.I. Osman, J. Bissoli, A.J. Bullock, C.R. Chapple, and S. MacNeil, Biomaterials for pelvic floor reconstructive surgery: how can we do better? Biomed Res Int 2015;2015:968087.
[382] Hillary, C.J., S. Roman, A.J. Bullock, N.H. Green, C.R. Chapple, and S. MacNeil, Developing Repair Materials for Stress Urinary Incontinence to Withstand Dynamic Distension. PLoS One 2016;11:e0149971.
[383] Park, S.A., I.A. Kim, Y.J. Lee, J.W. Shin, C.R. Kim, J.K. Kim, Y.I. Yang, and J.W. Shin, Biological responses of ligament fibroblasts and gene expression profiling on micropatterned silicone substrates subjected to mechanical stimuli. J Biosci Bioeng 2006;102:402-12.
[384] Eliason, B.J., M.M. Frisella, B.D. Matthews, and C.R. Deeken, Effect of repetitive loading on the mechanical properties of synthetic hernia repair materials. J Am Coll Surg 2011;213:430-5.
[385] McHugh, G.J., D.J. Wild, and J.H. Havill, Polyurethane central venous catheters, hydrochloric acid and 70% ethanol: a safety evaluation. Anaesth Intensive Care 1997;25:350-3.
[386] Crnich, C.J., J.A. Halfmann, W.C. Crone, and D.G. Maki, The effects of prolonged ethanol exposure on the mechanical properties of polyurethane and silicone catheters used for intravascular access. Infect Control Hosp Epidemiol 2005;26:708-14.
[387] Rubanyi, G.M., A. Johns, and K. Kauser, Effect of estrogen on endothelial function and angiogenesis. Vascul Pharmacol 2002;38:89-98.
[388] Song, W., X. Yu, D.C. Markel, T. Shi, and W. Ren, Coaxial PCL/PVA electrospun nanofibers: osseointegration enhancer and controlled drug release device. Biofabrication 2013;5:035006.
[389] Jiang, H., D. Fang, B. Hsiao, B. Chu, and W. Chen, Preparation and characterization of ibuprofen-loaded poly(lactide-co-glycolide)/poly(ethylene glycol)-g-chitosan electrospun membranes. J Biomater Sci Polym Ed 2004;15:279-96.

[390] Zeng, J., A. Aigner, F. Czubayko, T. Kissel, J.H. Wendorff, and A. Greiner, Poly(vinyl alcohol) nanofibers by electrospinning as a protein delivery system and the retardation of enzyme release by additional polymer coatings. Biomacromolecules 2005;6:1484-8.
[391] Ranganath, S.H. and C.H. Wang, Biodegradable microfiber implants delivering paclitaxel for post-surgical chemotherapy against malignant glioma. Biomaterials 2008;29:2996-3003.
[392] Maretschek, S., A. Greiner, and T. Kissel, Electrospun biodegradable nanofiber nonwovens for controlled release of proteins. J Control Release 2008;127:180-7.
[393] Blackwood, K.A., R. McKean, I. Canton, C.O. Freeman, K.L. Franklin, D. Cole, I. Brook, P. Farthing, S. Rimmer, J.W. Haycock, A.J. Ryan, and S. MacNeil, Development of biodegradable electrospun scaffolds for dermal replacement. Biomaterials 2008;29:3091-104.
[394] Hashizume, R., K.L. Fujimoto, Y. Hong, N.J. Amoroso, K. Tobita, T. Miki, B.B. Keller, M.S. Sacks, and W.R. Wagner, Morphological and mechanical characteristics of the reconstructed rat abdominal wall following use of a wet electrospun biodegradable polyurethane elastomer scaffold. Biomaterials 2010;31:3253-65.
[395] Salgaller, M.L. and P.K. Bajpai, Immunogenicity of glutaraldehyde-treated bovine pericardial tissue xenografts in rabbits. J Biomed Mater Res 1985;19:1-12.
[396] Petros, P., A basis for long-term midurethral tape complications. Neurourol Urodyn 2011;30:199-200.
[397] Blaivas, J.G., R.S. Purohit, M.S. Benedon, G. Mekel, M. Stern, M. Billah, K. Olugbade, R. Bendavid, and V. Iakovlev, Safety considerations for synthetic sling surgery. Nat Rev Urol 2015;12:481-509.
[398] Petros, P.E., U.I. Ulmsten, and J. Papadimitriou, The autogenic ligament procedure: a technique for planned formation of an artificial neo-ligament. Acta Obstet Gynecol Scand Suppl 1990;153:43-51.
[399] de Almeida, S.H., M.A. Rodrigues, E. Gregorio, J. Crespigio, and H.A. Moreira, Influence of sling material on inflammation and collagen deposit in an animal model. Int J Urol 2007;14:1040-3.
[400] Jones, R., P. Abrams, P. Hilton, K. Ward, and M. Drake, Risk of tape-related complications after TVT is at least 4%. Neurourol Urodyn 2010;29:40-1.
[401] Remes, A. and D.F. Williams, Immune response in biocompatibility. Biomaterials 1992;13:731-43.
[402] Wolf, M.T., C.L. Dearth, C.A. Ranallo, S.T. LoPresti, L.E. Carey, K.A. Daly, B.N. Brown, and S.F. Badylak, Macrophage polarization in response to ECM coated polypropylene mesh. Biomaterials 2014;35:6838-49.
[403] Kim, G.H., Electrospun PCL nanofibers with anisotropic mechanical properties as a biomedical scaffold. Biomed Mater 2008;3:025010.
[404] Brown, T.D., Techniques for mechanical stimulation of cells in vitro: a review. J Biomech 2000;33:3-14.
[405] Goldstein, S.R. and N. Nanavati, Adverse events that are associated with the selective estrogen receptor modulator levormeloxifene in an aborted phase III osteoporosis treatment study. Am J Obstet Gynecol 2002;187:521-7.