Dedication

I would like to dedicate this thesis to my loving wife, Fatima, who provided me with unconditional love and support throughout the course of my research.
Abstract

Stress urinary incontinence (SUI) and pelvic organ prolapse (POP) lead to significant interference in the quality of life of the millions of women affected by them. The treatment options for these women include surgical prostheses which are currently fraught with high failure and complication rates. Our aim was to explore tissue engineering as a solution to the problems of prosthetic failure. The objective was to identify suitable scaffolds that may be used to produce tissue engineered prostheses, with autologous fibroblasts, for use in women with SUI/POP. Seven candidate scaffolds; AlloDerm, cadaveric dermis, polypropylene, porcine dermis, sheep forestomach, porcine small intestinal submucosa and thermoannealed poly(l)lactic acid were investigated. We seeded 800 000 oral fibroblasts to 2cm² of each scaffold. We assessed the metabolic activity and proliferation of attached cells using AlamarBlue and DAPI staining, contraction using serial photographs, biomechanical properties using a uniaxial tensiometer, collagen production using Sirius red and immunofluorescence staining, and extracellular matrix production using scanning electron microscopy. In addition, the effect of mechanical restraint, simple variable stress and ascorbate-2-phosphate on the above parameters of the tissue engineered prostheses were also investigated. Two scaffolds; porcine small intestinal submucosa and thermoannealed poly(l) lactic acid have been identified as suitable matrices for supporting fibroblast attachment and new extracellular matrix production. Both scaffolds showed cells proliferated and increased their metabolic activity over 14 days of culture. Immunostaining also revealed new collagen I, III and elastin. The mechanical properties of the two scaffolds when cellularised were also close to those of native tissue. We have also shown that mechanical and chemical modulation of the culture environment may be beneficial in producing tissue engineered prostheses with improved properties. Further work will now take these findings into in vivo models.
Contents

DEDICATION.........................................................................................................................i

ABSTRACT..........................................................................................................................ii

CONTENTS...........................................................................................................................iii

PUBLICATIONS AND PRESENTATIONS..................................................................................Viii

ACKNOWLEDGEMENTS.......................................................................................................ix

ABBREVIATIONS................................................................................................................X

LIST OF FIGURES AND TABLES..........................................................................................Xi

Chapter 1  Introduction

1.1 Epidemiology of stress urinary incontinence  1
1.1.1 Aetiology of SUI  2
1.1.2 Risk factors for SUI  6
1.2 Epidemiology of pelvic organ prolapse  7
1.2.1 Aetiology of POP  11
1.2.2 Risk factors for POP  12
1.3 Treatment of SUI  13
1.3.1 Non surgical treatments  13
1.3.2 Surgical treatments  15
1.3.2.1 Complications of SUI surgery  20
1.3.2.2 Outcome measures of SUI surgery  21
1.4 Treatment of POP  22
1.4.1 Non surgical treatments  22
1.4.2 Surgical treatments  23
1.4.2.1 Upper vaginal prolapse  23
1.4.2.2 Anterior vaginal wall prolapse  24
1.4.2.3 Posterior vaginal wall prolapse  28
1.4.2.4 Complications of POP surgery  28
1.4.2.5 Outcome measures of POP surgery  29
1.5 Simultaneous SUI and POP  29
1.6 Prostheses in SUI/ POP  30
1.6.1 Absorbable synthetic prostheses  32
1.6.2 Non Absorbable synthetic prostheses  32
1.6.3 Autologous prostheses  33
1.6.4 Allograft prostheses  34
1.6.5 Xenograft prostheses  35
1.6.6 Tissue engineered prostheses  35
Chapter 2  Materials and Methods

2.1  Materials  76
2.2  Cell isolation and culture  77
2.3  Material preparation and cell seeding  78
2.4  AlamarBlue  78
2.5  DAPI  78
2.6  Contraction  79
2.7  Tensiometry  79
2.8  Sirius red  79
2.9  Immunofluorescence  80
2.10  Scanning electron microscopy  81
2.11  Restraint  81
2.12  Variable stress rig  81
2.13  Vitamin C  82
## Chapter 3  Attachment of fibroblasts to scaffolds

### 3.1 Introduction  85
### 3.2 AlamarBlue results  86
### 3.2.1 Alloderm  86
### 3.2.2 Cadaveric dermis  86
### 3.2.3 Polypropylene  86
### 3.2.4 Porcine dermis  87
### 3.2.5 Sheep forestomach  87
### 3.2.6 Small intestinal submucosa  87
### 3.2.7 Thermoannealed PLA  87
### 3.3 Comparison of cell metabolic activity on potential scaffold materials  92
### 3.4 DAPI images  92
### 3.5 Discussion  96

## Chapter 4  Comparison of the mechanical properties of scaffolds seeded with fibroblasts

### 4.1 Introduction  100
### 4.2 Tensiometry results  101
### 4.2.1 Alloderm  101
### 4.2.2 Cadaveric dermis  101
### 4.2.3 Polypropylene  101
### 4.2.4 Porcine dermis  101
### 4.2.5 Sheep forestomach  102
### 4.2.6 Small intestinal submucosa  102
### 4.2.7 Thermoannealed PLA  102
### 4.3 Comparison of materials  102
### 4.4 Discussion  106

## Chapter 5  Contraction of scaffolds seeded with fibroblasts

### 5.1 Introduction  108
### 5.2 Scaffold contraction results  109
### 5.2.1 Alloderm  109
### 5.2.2 Cadaveric dermis  109
### 5.2.3 Polypropylene  109
### 5.2.4 Porcine dermis  109
### 5.2.5 Sheep forestomach  109
### 5.2.6 Small intestinal submucosa  110
### 5.2.7 Thermoannealed PLA  110
### 5.3 Comparison of materials  110
### 5.4 Discussion  116
## Chapter 6  Matrix production by fibroblasts on scaffolds

6.1  Introduction 118  
6.2  Total collagen production results 118  
6.3  Immunostaining for collagen I, III, IV and elastin results 121  
6.4  Scanning electron microscopy results 127  
6.5  Discussion 130  

## Chapter 7  The effect of restraint on the production of tissue engineered prostheses

7.1  Introduction 133  
7.2  The effect of restraint on the production of tissue engineered prostheses results 134  
7.2.1  Cell attachment 134  
7.2.2  Tensiometry 134  
7.2.3  Contraction 140  
7.2.4  Matrix production 140  
7.3  Discussion 146  

## Chapter 8  The effect of a variable stress rig on a thermoannealed PLA tissue engineered prosthesis

8.1  Introduction 148  
8.2  The effect of variable stress results 149  
8.2.1  Cell attachment 149  
8.2.2  Contraction 153  
8.2.3  Tensiometry 153  
8.2.4  Matrix production 153  
8.3  Discussion 158  

## Chapter 9  The effect of Vitamin C on a PLA tissue engineered prosthesis

9.1  Introduction 160  
9.2  Results of the effect of Asc-2p 161  
9.2.1  Cell attachment 161  
9.2.2  Tensiometry 161  
9.2.3  Contraction 161  
9.2.4  Matrix production 161  
9.3  Discussion 167
Chapter 10  Discussion

10.1  Failure of current prostheses  169
10.2  Solutions for an improved prosthesis  182
10.3  Relating results from this study to the current literature  186
10.4  On-going and future work  193

Conclusions  196

References  197

Appendices

Appendix 1  Data collection tables of the biomechanical testing of pelvic organ prostheses

Appendix 2  Patient information leaflet for buccal mucosa samples
Publications


Presentations

Comparative investigation of seven candidate scaffolds for the production of an autologous tissue engineered connective tissue for use in SUI/POP. International Continence Society meeting, Glasgow.

Investigation of seven candidate scaffolds for the production of an autologous tissue engineered connective tissue for use in SUI/POP. Tissue and cell engineering meeting, Manchester.

The effect of scaffold restraint on the properties of tissue engineered prostheses being developed for use in SUI/POP. European Association of Urology congress, Vienna.

Engineering a novel tissue engineered autologous prosthesis for use in SUI/POP repair. British Association of Urological Surgeons academic meeting, Dublin.


Developing an autologous tissue engineered prosthesis for use in SUI/POP. International Continence Society meeting, Toronto.

A novel tissue engineering approach for creating prostheses for the treatment of SUI/POP. European Association of Urology congress, Barcelona.

Increasing collagen production & contraction with ascorbate in a tissue engineered autologous prosthesis for use in the treatment of SUI/POP. British Association of Urological Surgeons academic meeting, London.
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Finally, I would like to thank the Urology Foundation and the Robert Luff foundation for a two year research fellowship.

To all of the above, I extend my deepest appreciation.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AL</td>
<td>Alloderm*</td>
</tr>
<tr>
<td>Asc-2p</td>
<td>Ascorbate-2-phosphate</td>
</tr>
<tr>
<td>CD</td>
<td>Cadaveric dermis</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’,6-diamidino-2-phenylindole dihydrochloride</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EPAC</td>
<td>Electronic Personal Assessment Questionnaire</td>
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<tr>
<td>FACS</td>
<td>Fluorescence Activated Cell Sorting</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycans</td>
</tr>
<tr>
<td>HOXA11</td>
<td>Homeobox-A11</td>
</tr>
<tr>
<td>ICIQ-UF SF</td>
<td>International Consultation on Incontinence modular Questionnaire-Short Form</td>
</tr>
<tr>
<td>ICS</td>
<td>International Continence Society</td>
</tr>
<tr>
<td>LAMC1</td>
<td>Laminin-C1 gene</td>
</tr>
<tr>
<td>LUTS</td>
<td>Lower urinary tract symptoms</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MTS</td>
<td>3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute of Clinical Excellence</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCL</td>
<td>Polycaprolactone</td>
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<tr>
<td>PDGF</td>
<td>Platelet derived growth factor</td>
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<tr>
<td>PFDI</td>
<td>Pelvic Floor Distress Inventory</td>
</tr>
<tr>
<td>PFIQ</td>
<td>Pelvic Floor Impact Questionnaire</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly glycolic acid</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly(L)lactic acid</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly lactic co glycolic acid</td>
</tr>
<tr>
<td>POP</td>
<td>Pelvic organ prolapse</td>
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<tr>
<td>POPQ</td>
<td>Pelvic Organ Prolapse Quantification system</td>
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<tr>
<td>PPL</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>Roswell Park Memorial Institute 1640 medium</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SF</td>
<td>Sheep forestomach</td>
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<tr>
<td>SIS</td>
<td>Small intestinal submucosa</td>
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<tr>
<td>SUI</td>
<td>Stress urinary incontinence</td>
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<tr>
<td>Th PLA</td>
<td>Thermoannealed poly(L)lactic acid</td>
</tr>
<tr>
<td>TIMP</td>
<td>Tissue inhibitors of matrix metalloproteinases</td>
</tr>
<tr>
<td>TOT</td>
<td>Transobturator tape</td>
</tr>
<tr>
<td>TVT</td>
<td>Tension free vaginal tape</td>
</tr>
<tr>
<td>TVT-O</td>
<td>Tension free vaginal tape inside-out</td>
</tr>
<tr>
<td>UTS</td>
<td>Ultimate tensile strength</td>
</tr>
<tr>
<td>UT Strain</td>
<td>Ultimate tensile strain</td>
</tr>
<tr>
<td>YM</td>
<td>Young’s modulus of elasticity</td>
</tr>
</tbody>
</table>
List of figures and tables

Figures:

Fig 1.1.1.1  Diagram of the endopelvic fascia
Fig 1.1.1.2  Image of the pelvic fascia and ligaments
Fig 1.1.1.3  Diagram of the hammock theory for urethral support
Fig 1.2.1  Diagram of anterior compartment prolapse (cystocele)
Fig 1.2.2  Diagram of middle compartment vaginal prolapse (enterocele)
Fig 1.2.3  Diagram of posterior compartment prolapse (rectocele)
Fig 1.2.1.1  Diagram of the pelvic floor supports
Fig 1.3.2  Diagram of an anterior vaginal repair
Fig 1.3.2.2  Diagram of the colposuspension procedure
Fig 1.3.2.3  Variations of the sling/ tape procedures
Fig 1.3.2.4  Diagram of the anatomy of the TOT procedure
Fig 1.4.2.1.1  Diagram of the common positions of tapes in SUI/ POP procedures
Fig 1.4.2.2.1  Diagram of a paravaginal repair
Fig 1.4.2.2.2  Diagram of an anterior repair with graft placement
Fig 1.6.7.1  Schematic of the ideal tissue engineered prosthesis and its outputs
Fig 1.8.2.1  Image of fibroblasts
Fig 1.10.1  Stress Vs strain plot
Fig 1.10.4.1  Graph of the change of the UTS of prostheses pre and post explantation
Fig 1.10.4.2  Graph of the change in the YM of prostheses pre and post explantation
Fig 1.10.5.1  Graph of the YM and UTS of prostheses in relation to native tissue
Fig 1.10.5.2  Graph of the YM and UTS of prostheses post implantation in relation to native tissue
Fig 1.11.1  H & E stained image of a section through vagina and bladder wall with endopelvic fascia
Fig 1.11.2  H & E staining of vaginal tissue
Fig 1.11.1.1  Immunohistochemical staining for collagen I in women with and without SUI
Fig 1.11.1.2  Immunohistochemical staining for collagen III in women with and without SUI
Fig 1.11.2.1  Immunohistochemical staining for elastin in women with and without SUI
Fig 2.11.1  Picture of variable stress rig 1
Fig 2.11.2  Diagram of variable stress rig 2
Fig 3.2.1.1  Graph of the metabolic activity of fibroblasts on AL
Fig 3.2.2.1  Graph of the metabolic activity of fibroblasts on CD
Fig 3.2.3.1  Graph of the metabolic activity of fibroblasts on PPL
Fig 3.2.4.1  Graph of the metabolic activity of fibroblasts on PD
Fig 3.2.5.1  Graph of the metabolic activity of fibroblasts on SF
Fig 3.2.6.1  Graph of the metabolic activity of fibroblasts on SIS
Fig 3.2.7.1  Graph of the metabolic activity of fibroblasts on Th PLA
Fig 3.3.1  Graph of the metabolic activity of fibroblasts on the seven scaffolds
Fig 3.4.1  DAPI stained images cell nuclei on the seven scaffolds
Fig 4.3.1  Graph of the UTS of the seven scaffolds with and without cells
Fig 4.3.2  Graph of the UT strain of the seven scaffolds with and without cells
Fig 4.3.3  Graph of the YM of the seven scaffolds with and without cells
Fig 5.2.1  Graph of the % contraction of AL over 14 days culture
Fig 5.2.2  Graph of the % contraction of CD over 14 days culture
Fig 5.2.3  Graph of the % contraction of PPL over 14 days culture
Fig 5.2.4  Graph of the % contraction of PD over 14 days culture
Fig 5.2.5  Graph of the % contraction of SF over 14 days culture
Fig 5.2.6  Graph of the % contraction of SIS over 14 days culture
Fig 5.2.7  Graph of the % contraction of Th PLA over 14 days culture
Fig 5.3.1 Graph of the % contraction of the seven scaffolds with cells over 14 days culture
Fig 6.2.1 Graph of the total collagen production by fibroblasts on the seven scaffolds
Fig 6.3.1a DAPI and FITC stained image of collagen I on Th PLA
Fig 6.3.1b DAPI and FITC stained image of collagen III on Th PLA
Fig 6.3.1c DAPI and FITC stained image of elastin on Th PLA
Fig 6.3.2a DAPI and FITC stained image of collagen I on SIS
Fig 6.3.2b DAPI and FITC stained image of collagen III on SIS
Fig 6.3.4 Graph of the mean collagen I scores on the seven scaffolds
Fig 6.3.5 Graph of the mean collagen III scores on the seven scaffolds
Fig 6.3.6 Graph of the mean elastin scores on the seven scaffolds
Fig 6.4.1 Graph of the mean ECM scores on the seven scaffolds
Fig 6.4.2 SEM images of the seven scaffolds with and without cells
Fig 7.2.1.1 Graph of the metabolic activity of fibroblasts on the seven restrained scaffolds over 14 days culture
Fig 7.2.1.2 Graph of the metabolic activity of fibroblasts on the seven scaffolds with and without restraint at 14 days culture
Fig 7.2.1.3 DAPI images of the distribution of fibroblasts on restrained and unrestrained PPL
Fig 7.2.2.1 Graph of the UTS of the seven restrained scaffolds with and without cells
Fig 7.2.2.2 Graph of the UT strain of the seven restrained scaffolds with and without cells
Fig 7.2.2.3 Graph of the YM of the seven restrained scaffolds with and without cells
Fig 7.2.2.4 Graph of the UTS of the seven scaffolds with and without restraint
Fig 7.2.2.5 Graph of the UT strain of the seven scaffolds with and without restraint
Fig 7.2.2.6 Graph of the YM of the seven scaffolds with and without restraint
Fig 7.2.3.1 Graph of the % contraction of restrained and unrestrained scaffolds with and without cells after 14 days culture
Fig 7.2.4.1.1 Graph of the collagen produced by scaffolds with and without restraint
Fig 7.2.4.2.1 Graph of the mean collagen I scores on the seven scaffolds with and without restraint
Fig 7.2.4.2.2 Graph of the mean collagen III scores on the seven scaffolds with and without restraint
Fig 7.2.4.2.3 Graph of the mean elastin scores on the seven scaffolds with and without restraint
Fig 7.2.4.3.1 Graph of the mean ECM scores on the seven scaffolds with and without restraint
Fig 7.2.4.3.2a SEM image of unrestrained PPL
Fig 7.2.4.3.2.b SEM image of restrained PPL
Fig 8.1.1 Diagram of the variable stress on the female pelvis
Fig 8.1.2 Diagram of the variable stress rig
Fig 8.2.1 Graph of the metabolic activity of fibroblasts on Th PLA in variable stress rig 1
Fig 8.2.1.1 Graph of the metabolic activity of fibroblasts on Th PLA in variable stress rig 2
Fig 8.2.1.2 Images of DAPI stained nuclei on Th PLA in variable stress rig 2
Fig 8.2.3.1 Graph of the UTS of Th PLA scaffolds in variable stress rig 2
Fig 8.2.3.2 Graph of the UT strain of Th PLA scaffolds in variable stress rig 2
Fig 8.2.3.3 Graph of the UTS of Th PLA scaffolds in variable stress rig 2
Fig 8.2.4.1 Graph of the collagen production by cells on Th PAL in variable stress rig 2
Fig 8.2.4.2 Immunostained images of collagen I on Th PLA on variable stress rig 2
Fig 8.2.4.3 Immunostained images of elastin on Th PLA on variable stress rig 2
Fig 9.2.1.1 Graph of the metabolic activity of fibroblasts on PLA in varying concentrations of Asc-2p over 14 days culture
Fig 9.2.1.2  DAPI stained images of nuclei on PLA scaffolds in varying concentrations of Asc-2p
Fig 9.2.2.1  Graph of the UTS of PLA scaffolds with cells cultured in varying concentrations of Asc-2p
Fig 9.2.2.2  Graph of the UT strain of PLA scaffolds with cells cultured in different concentrations of Asc-2p
Fig 9.2.2.3  Graph of the YM of PLA scaffolds with cells cultured in different concentrations of Asc-2p
Fig 9.2.3.1  Graph of the % contraction of PLA scaffolds in different concentration of Asc-2p
Fig 9.2.4.1  Graph of the total collagen production by fibroblasts on PLA scaffolds in different concentrations of Asc-2p
Fig 10.1.1  Timeline of the failure and complications of synthetic prostheses for SUI
Fig 10.1.2  Timeline of the failure and complications of autologous sling procedures for SUI
Fig 10.1.3  Timeline for the failure and complications of biological grafts for SUI
Fig 10.1.4  Schematic of the host inflammatory response to synthetic implants
Fig 10.1.5  Schematic of the host inflammatory response to autologous transplants
Fig 10.1.6  Schematic of the host inflammatory response to biological grafts
Fig 10.2.1  Schematic of the target host inflammatory response to tissue engineered prostheses
Fig 10.3.1  Schematic of the proposed one stage approach
Fig 10.4.1  Schematic of the future work with tissue engineered prostheses

Tables:
Table 1.2.1.1  Structural elements of pelvic organ support
Table 1.10.2.1  Biomechanical properties of paravaginal tissue
Table 1.10.3.1  Biomechanical properties of pelvic organ prostheses
Table 1.10.4.1  Biomechanical properties of pelvic organ prostheses post explantation
Table 1.11.7.1  Summary of the evidence reporting changes in connective tissue components in women with SUI/ POP
Table 1.11.7.2  Summary of the evidence reporting mRNA changes in connective tissue components in women with SUI/ POP
Table 3.1.1.  Parameters of the seven scaffolds
Table 3.3.1  Order of materials according to cell attachment
Table 4.3.1  Order of materials according to UTS
Table 4.3.2  Order of materials according to UT strain
Table 4.3.3  Order of materials according to YM
Table 5.3.1  Order of materials according to contraction
Table 6.2.1  Order of materials according to total collagen production
Table 6.3.1  Summary of immunostaining scores for collagen I, III IV and elastin
Table 6.4.1  Summary of SEM results of ECM production
Table 7.2.3.1  Differences in contraction of the seven scaffolds with and without restraint
Table 7.2.4.2.1  Quantitative and qualitative results of immunostaining for collagen I, III and elastin with and without restraint
Table 7.2.4.3.1  Quantitative and qualitative results of ECM production with and without restraint
Table 10.3.1  Summary of the relative properties of scaffolds in different conditions