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 $2cm^2$ 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 in to *in vivo* models.

Contents

Publications

Mangera A, Bullock AJ, Chapple CR, MacNeil S. Are biomechanical properties predictive of the success of prostheses used in stress urinary incontinence and pelvic organ prolapse? A systematic review. Neurourol Urodyn. 2012 Jan;31(1):13-21.

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 a tissue engineered autologous prosthesis for use in SUI/POP repair; which scaffold? Biomaterials and Tissue Engineering Group meeting, Leeds.

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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Abbreviations

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

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