Augmentation Strategies in Rotator Cuff Repair

A dissertation submitted to the University of Oxford in partial fulfilment of the requirements for the degree of

Doctor of Philosophy



Mustafa Saad Rashid

Worcester College

Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences

University of Oxford

Trinity Term 2018

Supervisors: Professor Andrew Carr, Associate Professor Stephanie Dakin, and Dr. Sarah Snelling

Declaration

I certify that this thesis is a true and honest account of my own research, which was conducted ethically and the results obtained are genuine. Where I have drawn on the work, ideas, and results of others, this has been appropriately acknowledged in the thesis.

Mustafa Saad Rashid

Mustafa S. Rashid
Worcester College

Abstract

Rotator cuff repair surgery is commonly performed for symptomatic tears failing to resolve with conservative measures. This thesis investigated the healing rate following rotator cuff repair, and the influence of age and tear size. Patient tissue responses to 2 commercially available biological patches used in cuff repair were subsequently investigated. Finally, safety and efficacy of 2 novel biodegradable polymeric scaffolds to repair an ovine tendon defect, was investigated.

The overall rate of healing following rotator cuff repair was 58% at 1 year. Healing was influenced by age and tear size, however age was the predominant prognostic factor. A predictive model for healing was generated, demonstrating likelihood of healing as low as 17%. Patient supraspinatus tendon biopsies demonstrated an adverse response to dermal allograft and xenograft patches currently used in cuff repair augmentation, whereby significant extracellular matrix disruption was observed. One patient receiving the xenograft experienced a significant adverse immune response, characterised by extensive infiltration of pro-inflammatory cells. A novel biodegradable polydioxanone / polycaprolactone patch was investigated in an ovine tendon injury model. At 3 months, this patch induced tendon healing, without adverse systemic or tissue responses. Significant fibroblast infiltration and neovascularisation was observed within the scaffold. A biodegradable polydioxanone multifilament suture was also

developed. This novel suture demonstrated bioactivity, inducing tendon healing without adverse systemic or tissue responses. Fibroblast infiltration and neovascularisation was observed when compared to monofilament polydioxanone control suture.

Healing following cuff repair remains a challenge. Current biological patches used to augment repair are not supported by clinical efficacy trials, and demonstrated a deleterious effect on the native tendon. Novel synthetic implants tailored for tendon repair successfully induced tendon healing in an ovine model. Future work will explore the safety and clinical efficacy of these implants for rotator cuff repair augmentation in humans.

Acknowledgements

I dedicate this thesis to my wife, Malin. It would not have been conceivable to reach this point in my life and career without your support and encouragement. Secondly, my mother, Shetha. You showed me what was possible with hard work and commitment.

This thesis would have not been possible without the support, guidance, and encouragement of my supervisors, Andy, Steph, and Sarah. Thank you for everything you have done for me over the past 3 years. I am forever grateful. There are many people at Oxford that have helped me on my DPhil journey. It is difficult to really appreciate how their many hours of discussion, advice, and friendship has shaped this thesis, and the scientist that I became during this time. Thank you Rich, Ed, Nav, Wataru, Mat, Jolet, Mike, Jo, Tonia, Hayley, Pierre, Cameron, Kim, Bridget, Jonathan, Roger, Jay, Roberta, Ben, Lucy, Ann, and Afsie.

Contents

Chapter 1: Rotator cuff tendon tears.....24

1.1 Human shoulder joint.....24

1.2 Human rotator cuff anatomy.....25

1.2.1 Human rotator cuff tendon structure.....28

1.2.2 Human enthesis.....31

1.3 Function of the human rotator cuff.....32

1.4 Rotator cuff tendon tears.....34

1.4.1 Rotator cuff tear assessment.....35

1.4.2 Symptoms of rotator cuff tear.....37

1.4.3 Theories of rotator cuff tear aetiology.....38

1.4.4 Natural history of rotator cuff tendon tears.....42

1.5 Rotator cuff repair.....45

1.5.1 Evolution of rotator cuff repair techniques.....46

1.5.2 Evolution of implants used in rotator cuff repair.....49

1.5.3 Assessing outcome following rotator cuff repair.....52

1.5.4 Healing rate following rotator cuff repair.....60

1.6 Augmentation of rotator cuff repair.....67

1.7 Unmet clinical needs related to rotator cuff tears.....76

1.8 Overall thesis aims and objectives.....79

1.8.1 Thesis chapter aims and hypotheses.....79

Chapter 2: The influence of age and tear size on rotator cuff healing rate.81

2.1 Outcomes of rotator cuff repair surgery.....81

Contents

2.2 Surgical goal of rotator cuff surgery 82

2.2.1 Does tendon healing influence patient reported outcomes? 82

2.2.2 Healing rate after rotator cuff repair surgery 83

2.3 Aims 84

2.3.1 Hypotheses 85

2.4 Materials and methods 85

2.4.1 Patients recruited for the study 85

2.4.2 Surgical procedure and post-operative rehabilitation 89

2.4.3 Tear size assessment 90

2.4.4 Statistical analysis 90

2.5 Results 91

2.5.1 Failure rate by tear size 91

2.5.2 Relationship between age and tear size 92

2.5.3 Influence of age on healing rate 92

2.5.4 Influence of tear size on healing rate 92

2.5.5 Estimated probability of healing 93

2.6 Discussion 94

2.6.1 Reported healing rate by publication date 95

2.6.2 Age and tear size influence healing rate 95

2.6.3 Limitations 97

2.7 Conclusions 98

2.8 Biological considerations for rotator cuff healing 99

Contents

2.8.1 Observations in biological changes in rotator cuff tears 99

2.8.2 Rotator cuff repair augmentation 100

Chapter 3: Biological patch augmentation in rotator cuff repair 101

3.1 Tendon repair augmentation 101

3.1.1 Tendon repair with patch augmentation 102

3.1.2 Biological patch philosophy 103

3.1.3 GraftJacket patch 103

3.1.4 Permacol patch 104

3.1.5 Issues with porcine xenograft patches 104

3.2 Aim 105

3.2.1 Hypothesis 105

3.3 Materials and Methods 106

3.3.1 Rotator cuff repair surgery 108

3.3.2 Rehabilitation and follow-up 108

3.3.3 Tendon sample harvesting 108

3.3.4 Tendon sample handling and processing 109

3.3.5 Histology and immunohistochemistry 110

3.3.6 Microscopy, scoring, and image analysis 112

3.3.7 CellProfiler pipeline for quantitative analysis of IHC 115

3.4 Results 116

3.4.1 Histology results 120

3.4.2 Immunohistochemistry results 122

Contents

3.5 Discussion 126

3.5.1 Biomechanical properties of GraftJacket and Permacol patches 127

3.5.2 In vitro investigations of GraftJacket and Permacol 127

3.5.3 In vivo observations using GraftJacket and Permacol patches 129

3.5.4 Clinical studies using GraftJacket and Permacol in rotator cuff repair 130

3.6 Limitations 131

3.7 Conclusions 132

3.8 Biological versus synthetic patches for augmentation 133

3.8.1 Novel synthetic patches providing biophysical cues 134

Chapter 4: Biodegradable Electrospun Patch for Tendon Augmentation. 135

4.1 Current synthetic patches 135

4.1.1 Desirable properties of patches for tendon repair 136

4.2 Electrospinning technology 138

4.2.1 Development of an electrospun PDO/PCL patch 140

4.2.2 In vitro and in vivo studies of electrospun PDO/PCL patch 140

4.3 Animals models for tendon repair 141

4.4 Aims 141

4.4.1 Hypotheses 141

4.5 Materials and methods 142

4.5.1 Patch manufacturing and assembly 142

4.5.2 Production of electrospun polymeric layers 142

Contents

4.5.3 Weaving 143

4.5.4 Patch assembly and cutting 143

4.5.5 Scanning Electron Microscopy (SEM) 144

4.5.6 Surgical procedures and macroscopic observations 144

4.5.7 Euthanasia and tendon sample harvest 146

4.5.8 Haematology and serum inflammatory markers 147

4.5.9 Histology 147

4.5.10 Immunohistochemistry 149

4.5.11 Statistical methods 150

4.6 Results 151

4.6.1 Scaffold morphology of electrospun patches 151

4.6.2 Macroscopic observations of electrospun scaffold implanted tendons 153

4.6.3 Haematology and serum inflammatory markers of scaffold implanted sheep 155

4.6.4 Histology of electrospun scaffold implanted tendons 157

4.6.5 Immunohistochemistry 161

4.6.6 Adverse events 164

4.7 Discussion 164

4.7.1 Cellular infiltration into electrospun scaffolds 165

4.7.2 Neovascularisation of electrospun scaffolds 166

4.7.3 Foreign body giant cell response 168

Contents

4.7.4 Immune cell response to electrospun scaffolds....168

4.7.5 Strengths and limitations....169

4.8 Conclusions....171

4.9 Clinical implications of electrospun PDO/PCL patch for rotator cuff augmentation....172

4.10 The evolving rotator cuff repair construct....173

4.10.1 Innovation in sutures....174

Chapter 5: Multifilament biodegradable electrospun sutures....176

5.1 Rotator cuff repair construct....176

5.1.1 Failure following rotator cuff repair....178

5.2 Development of electrospun PDO sutures....179

5.3 Aims....180

5.3.1 Hypotheses....180

5.4 Materials and methods....181

5.4.1 Suture manufacture and characterisation....181

5.4.2 Preparation of the electrospinning solution....181

5.4.3 Electrospinning filaments....181

5.4.4 Filament drawing and multifilament assembly....182

5.4.5 Annealing and sterilisation....182

5.4.6 Scanning electron microscopy (SEM)....182

5.4.7 Surgical procedures....183

5.4.8 Necropsy and macroscopic observations....183

Contents

5.4.9 Haematology and serum inflammatory markers.....186

5.4.10 Histology 186

5.4.11 Statistical analysis.....187

5.5 Results.....187

5.5.1 Electrospun (ES) suture characterisation.....187

5.5.2 Macroscopic observations.....189

5.5.3 Haematology and serum inflammatory markers.....191

5.5.4 Histology results.....194

5.5.5 Adverse events 198

5.6 Discussion 200

5.6.1 Cellular infiltration into electrospun PDO sutures.....200

5.6.2 Neovascularisation of tissue integrated into electrospun PDO sutures 202

5.6.3 Understanding adverse events of electrospun PDO sutures.....203

5.6.4 Limitations.....204

5.7 Conclusions 205

5.8 Future work.....206

5.9 Clinical implications 208

5.9.1 Electrospun sutures vs electrospun patches for tendon augmentation 209

Chapter 6: General discussion 211

6.1 Rotator cuff healing.....211

Contents

6.1.1 Determining the healing rate following rotator cuff repair 213

6.1.2 Factors influencing healing rate 213

6.1.3 Response of native supraspinatus tendon to biological patches for augmentation 215

6.1.4 Response of tendon injury to electrospun synthetic patches for augmentation 217

6.1.5 Response of tendon injury to electrospun synthetic sutures for repair 218

6.2 Implications of this work.....219

6.2.1 Patients with a rotator cuff tear.....220

6.2.2 Surgeons treating rotator cuff tears.....221

6.2.3 Future use of biological patches to augment rotator cuff repair 222

6.2.4 Development of electrospun synthetic scaffolds to augment rotator cuff repair.....223

6.3 Future work.....224

6.4 Limitations of this thesis.....227

6.4.1 Defining rotator cuff healing 227

6.4.2 Recruitment for biological patch augmentation study.....228

6.4.3 Tissue biopsy following cuff repair 229

6.4.4 Tenocyte specific markers 230

6.4.5 Animal models for rotator cuff healing.....230

6.4.6 Control patch in tendon repair.....231

6.5 Key findings of this thesis 232

Contents

Appendices.....261

Appendix 1: Reprint of Oxford Shoulder Score (OSS).....261

List of Figures

Figure 1-1	Illustrations of the rotator cuff muscles	**27**
Figure 1-2	Anatomical dissection demonstrating rotator cuff bony footprint	**28**
Figure 1-3	Tendon hierarchical structure	**29**
Figure 1-4	Layers of the human rotator cuff	**31**
Figure 1-5	Human rotator cuff enthesis	**33**
Figure 1-6	Rotator cuff tear patterns	**36**
Figure 1-7	Rotator cuff repair constructs	**49**
Figure 1-8	Synthetic multifilament braided suture and suture tape used in rotator cuff repair	**53**
Figure 1-9	Reported rotator cuff healing in clinical trials, grouped by year of publication	**64**
Figure 1-10	Patch augmentation constructs	**69**
Figure 2-1	Flowchart of patients included in final analysis with full thickness rotator cuff tears treated with surgical repair in the UKUFF trial	**90**
Figure 2-2	Model for predicted rotator cuff healing rate at 1 year.	**94**
Figure 3-1	Scanning Electron Microscopy (SEM) images of GraftJacket patch, Permacol patch, and normal human supraspinatus tendon.	**104**
Figure 3-2	Study flowchart illustrating patient group allocation, follow-up at 4 weeks, and tissue analysis	**108**

List of Figures

Figure 3-3	Illustrations demonstrating needle biopsy site performed at the 4-week post-operative time point.	**110**
Figure 3-4	Representative H&E stained sections of torn supraspinatus tendon tissue sections	**114**
Figure 3-5	Example of cellularity count from a H&E stained section using ImageJ software	**115**
Figure 3-6	Representative images from CellProfiler pipeline	**117**
Figure 3-7	Representative histology showing tissue response to patch augmentation compared to control (no patch) group	**122**
Figure 3-8	Quantitative analysis of histology scoring comparing patch augmentation groups to control.	**123**
Figure 4-1	Electrospun PDO/PCL patch augmentation in ovine tendon injury model	**147**
Figure 4-2	Electrospun PDO/PCL patch composition and characterisation	**153**
Figure 4-3	Digital photograph images showing harvested tendons augmented with patch scaffolds	**156**
Figure 4-4	Histology of patch augmented ovine tendons	**160**
Figure 4-5	Immunohistochemical staining of patch augmented ovine tendons	**163**
Figure 4-6	Immunohistochemical staining for collagen proteins in patch augmented tendon sections	**164**

List of Figures

Figure 5-1	Rotator cuff repair constructs	**179**
Figure 5-2	Implantation of electrospun and monofilament sutures to augment tendon repair in an ovine model	**186**
Figure 5-3	Production and characterisation of electrospun PDO suture	**189**
Figure 5-4	Macroscopic appearance of repaired ovine tendons using electrospun PDO suture and extruded monofilament PDO suture	**192**
Figure 5-5	Histological appearance of ovine electrospun suture implanted tendons	**196**
Figure 5-6	Cellularity and vascularity of electrospun suture implanted tendons	**198**
Figure 5-7	Harvested ovine tendons with retained knots	**200**
Figure 5-8	Example of possible trial design for assessing clinical efficacy between electrospun implants used in rotator cuff repair	**211**
Figure 6-1	Summary of key findings of thesis chapters	**236**

List of Tables

Table 1-1	Extrinsic versus intrinsic theories of rotator cuff tear initiation highlighting key features of both proposed theories.	**43**
Table 1-2	Commonly used patient reported outcome measures for shoulder pathology related to the rotator cuff.	**55**
Table 1-3	Risk of bias assessment of Randomised Controlled Trials.	**62**
Table 1-4	Studies reporting the influence of age and tear size on rotator cuff repair healing as assessed by imaging post-surgery.	**66**
Table 1-5	Studies utilising patch augmentation in rotator cuff repair	**71**
Table 2-1	Inclusion and exclusion criteria for enrollment into the UKUFF trial.	**88**
Table 3-1	List of primary antibodies used in immunohistochemistry (IHC), including details and rationale for use.	**112**
Table 3-2	Study participants.	**119**
Table 4-1	Synthetic patches in clinical use for rotator cuff augmentation including manufacturer and polymer substrate.	**137**

List of Tables

Table 4-2	Primary antibodies details used in immunohistochemistry staining for patch implanted ovine tendons.	**151**
Table 4-3	Results of circumference measurements (in cm) of the right forelimb of the sheep pre-surgery and 3 months post-surgery.	**154**
Table 4-4	Results of full blood count and serum inflammatory markers taken pre-surgery and pre-necropsy / 3 months post-surgery.	**157**
Table 4-5	Intra and inter-observer reliability for histology scoring system.	**161**
Table 5-1	Results of circumference measurements (in cm) of the right forelimb of the sheep taken above and below the ergot pre-surgery and pre-necropsy / 3 months post-surgery.	**190**
Table 5-2	Results of full blood count and serum inflammatory markers taken pre-surgery and pre-necropsy / 3 months post-surgery.	**194**
Table 5-3	Comparison of advantages of utilising electrospun PDO sutures and electrospun PDO/PCL patches in rotator cuff repair surgery.	**210**

Abbreviations

AJC	Andrew J. Carr
AP	Anterior-Posterior
AZ	Arizona
BMP7	Bone Morphogenic Protein 7
CA	California
CD206	Cluster of Differentiation 206
CD4	Cluster of Differentiation 4
CD45	Cluster of Differentiation 45
CD68	Cluster of Differentiation 68
CI	Confidence Interval
CT	Computed Tomography
CTA	Computed Tomographic Arthrogram
DAB	3,3'-Diaminobenzidine
DDFT	Deep Digital Flexor Tendon
ECM	Extracellular Matrix
EQ-5D	European Quality of Life – 5 Domains
ES	Electrospun
FBGC	Foreign Body Giant Cell
FBGCC	Foreign Body Giant Cell Count
FCC	Fibroblast Cell Count
FDA	The Food and Drug Administration
FTT	Full thickness tear
g/L	Grams per Litre

Abbreviations

H&E	Haematoxylin and Eosin
HFIP	Hexafluoroisopropanolol
HMDI	4,4'-Diisocyanato-methylenedicyclohexane
ICC	Immune Cell Count
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IMS	Industrial Methylated Spirit
IN	Indiana
iNOS	Inducible Nitric Oxide Synthase
IQR	Interquartile Range
IRF5	Interferon Regulatory Factor 5
IVC	Inferior Vena Cava
kg	Kilogram
MA	Massachusetts
MCPJ	Metacarpophalangeal Joint
MF	Monofilament
mg/L	Milligram per Litre
MHRA	Medicines and Healthcare products Regulatory Agency
MRA	Magnetic Resonance Arthrogram
MRI	Maganetic Resonance Imaging
NHS	National Health Service
NJ	New Jersey
NT	Normal Tendon

Abbreviations

OR	Odds Ratio
OSS	Oxford Shoulder Score
P4HB	Poly-4-hydroxybutyrate
PCL	Polycaprolactone
PDO	Polydioxanone
PDPN	Podoplanin
PEEK	Polyether ether ketone
PLGA	Poly Lactic-co-Glycolic Acid
PROMs	Patients Reported Outcome Measures
PRP	Platelet Rich Plasma
PTT	Partial thickness tear
PUR	Polyurethane
RBC	Red Blood Cell
RC	Rotator Cuff
RCR	Rotator Cuff Repair
RCT	Rotator Cuff Tear
Ref	Reference
RTM	Regenerative Tissue Matrix
RVC	Royal Veterinary College
SDFT	Superficial Digital Flexor Tendon
SEM	Scanning Electron Microscopy
SIS	Small Intestine Submucosa
TE	Tennessee

Abbreviations

TGFβ	Transforming Growth Factor Beta
UK	United Kingdom
UKUFF	United Kingdom Rotator Cuff Trial
US	Ultrasound
USA	United States of America
USS	Ultrasound Scan
VAS	Visual Analogue Scale
α-SMA	Alpha Smooth Muscle Actin

Chapter 1: Rotator cuff tendon tears

This introductory chapter will outline the anatomical, clinical, and scientific challenges surrounding rotator cuff tendon tears. An overview of the normal shoulder and rotator cuff will be presented. This is followed by sections describing rotator cuff tendon tears, including their aetiology, epidemiology, clinical features, and management. Focussing on surgical repair of rotator cuff tendon tears, the evolution in surgical technique and implants will be discussed. Assessment of outcomes following surgical repair will be discussed, with a focus on determining healing, including its impact on pain and function. After outlining the relationship between healing and functional outcomes, the scientific literature will be summarised in relation to the reported healing rate and factors considered to influence this. As will be discussed later, there is significant dissonance regarding the reported healing rate. This has led some clinicians and researchers to develop augmentation strategies to improve the healing rate. These will be outlined, and a summary of scientific studies evaluating their clinical use will be presented. Finally, to conclude the literature review surrounding rotator cuff repair and augmentation, the unmet clinical needs of future research will be discussed. This final section will focus on the research areas that this thesis will cover, including the thesis aims and hypotheses.

1.1 Human shoulder joint

The human shoulder is a complex synovial joint that permits a wide range of motion in multiple planes. The glenohumeral joint is the main articulation of the

shoulder. The humeral head articular surface has approximately twice the radius of curvature of the glenoid fossa articular surface, and far less constrained than other synovial joints[1]. As such, this articulation relies on soft tissue static and dynamic stabilisers. One key feature of the glenohumeral joint is the rotator cuff tendon. This cuff of tissue is made up of contributions from four muscles. These muscles originate from the scapula and insert onto the humeral head. The anatomy and function of the rotator cuff is outlined below.

1.2 Human rotator cuff anatomy

The rotator cuff tendon in humans is made up of the confluence of four tendons that connect muscles originating from the scapula to the humeral head. These tendons form a cuff of tissue that is continuous from the posteroinferior portion of the humeral head to the anteroinferior portion. A rotator interval divides the posterior-superior cuff from the anterosuperior cuff as the long head of biceps enters the glenohumeral joint. The muscles that provide tendons forming the rotator cuff, from anterior to posterior, are subscapularis, supraspinatus, infraspinatus, and teres minor.

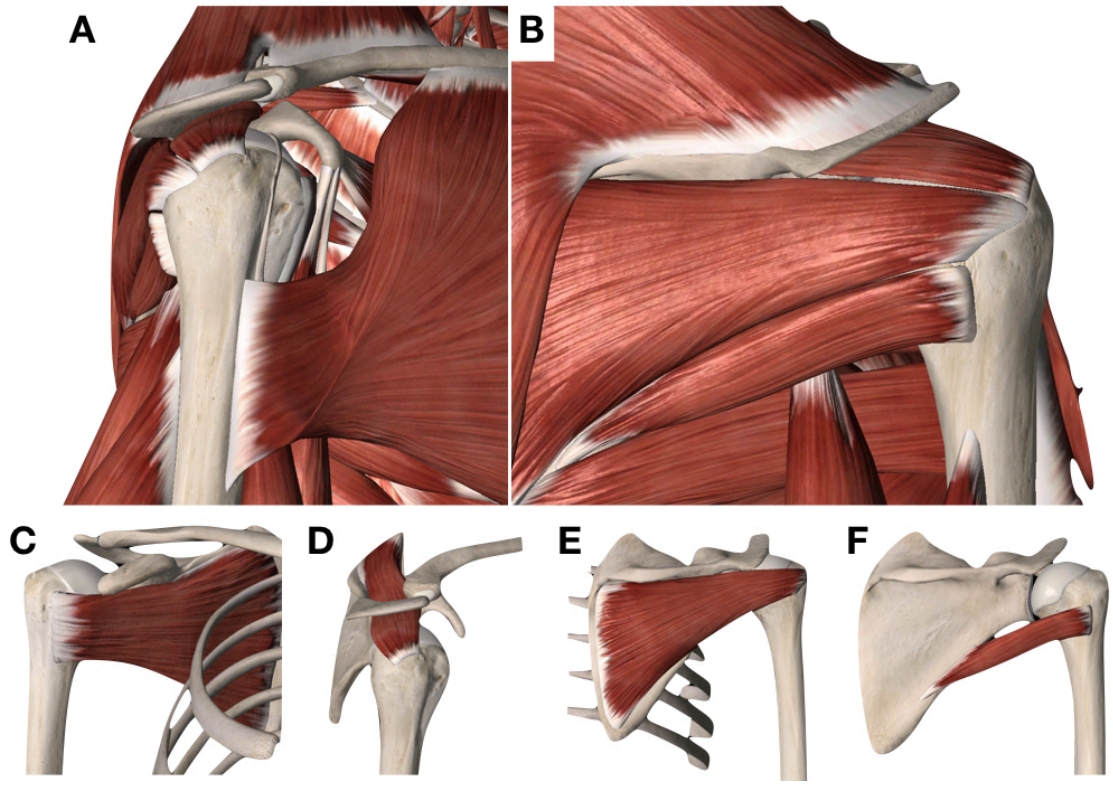


Figure 1-1: Illustrations of the rotator cuff muscles. Adapted from Essential Anatomy 5 app (3D4Medical, Dublin, Ireland). A: Lateral view of the rotator cuff muscles. B: Posterior view of the rotator cuff muscles. C-F: illustrations of the individual rotator cuff muscles (C- subscapularis, D- supraspinatus, E- infraspinatus, and F- teres minor).

Subscapularis originates from the anterior face of the scapula (subscapularis fossa) and inserts onto the lesser tuberosity of the humeral head. Supraspinatus originates from the supraspinatus fossa of the scapula and inserts onto the anterosuperior portion of the greater tuberosity of the humeral head. Infraspinatus originates from the posterior face of the scapula below the spine of the scapula (infraspinatus fossa) and inserts onto the posterosuperior portion of the greater tuberosity. Teres minor originates from the upper two thirds of the

lateral border of the scapula and inserts onto the posteroinferior portion of the greater tuberosity. The long head of biceps enters the synovial environment of the glenohumeral joint, running in the bicipital groove in the anterolateral aspect of the proximal humerus, to attach on the supraglenoid tubercle. The long head of biceps is not part of the rotator cuff but does separate the subscapularis tendon anteriorly from the rest of the rotator cuff tendons posteriorly.

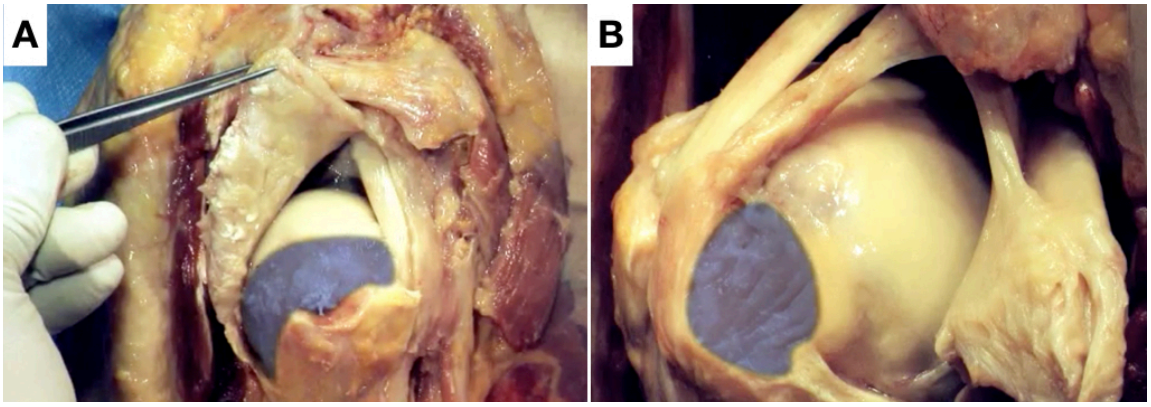


Figure 1-2: Anatomical dissection demonstrating rotator cuff bony footprint. Screenshot of anatomy demonstration video by George Lajtai (published on vumedi.com)[2]. A: anatomical footprint of the posterosuperior rotator cuff muscles (supraspinatus, infraspinatus, and teres minor) highlighted in blue. The long head of biceps tendon can be seen anterior (right) of the highlighted area. B: anatomical footprint of subscapularis tendon of the rotator cuff highlighted in blue.

1.2.1 Human rotator cuff tendon structure

Human tendons are composed of cells, and extracellular matrix (ECM). Fibroblasts are the predominant cell type in tendon tissue. The ECM is comprised of mainly type I collagen arranged in fibrils. Fibrils are bundled into fibres that have a characteristic crimp morphology. Fibres are in turn arranged in fascicles (secondary fibre bundle). Several fascicles combine to form tertiary fibre bundles.

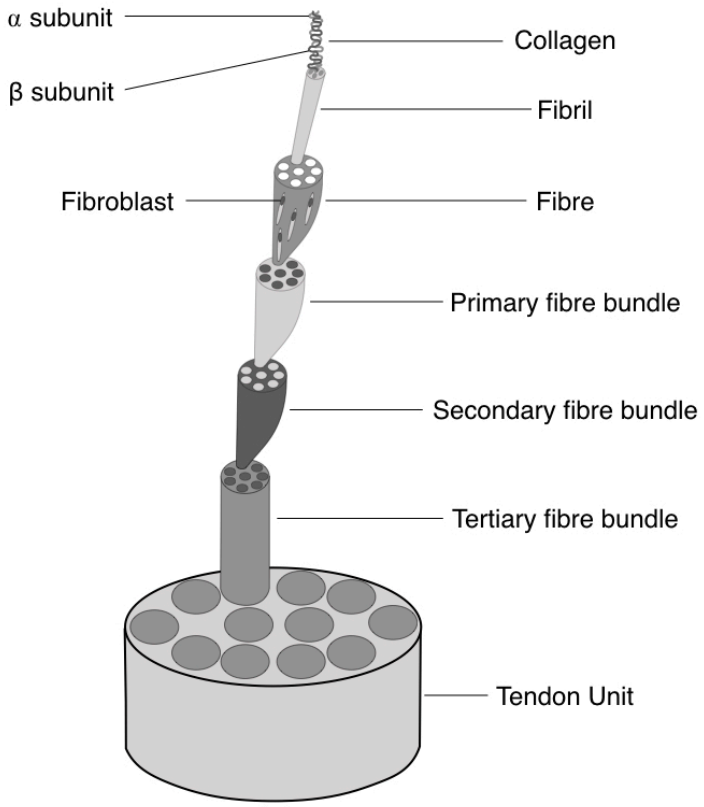


Figure 1-3 Tendon hierarchical structure. Illustration demonstrates how collagen fibrils are arranged in fibres, which are in turn arranged into bundles (primary, secondary, and tertiary), and fascicles.

The human rotator cuff can be divided into 5 layers based on histological sections[3]. Layer 1 includes the most superficial fibres, made up of the coracohumeral ligament, and covers the second layer, comprised of the fibres of the supraspinatus and infraspinatus tendons. This layer consists of tightly packed collagen fibres in parallel, arranged in large bundles. The third layer is made up of smaller collagen fascicles that are less orientated that in layer 2. Layer 4 consists of loose connective tissue made up of thick collagen bands. The deepest layer, layer 5, is thin and made up of continuous interwoven collagen fibrils that insert into the humeral bone via penetrating fibres of Sharpey.

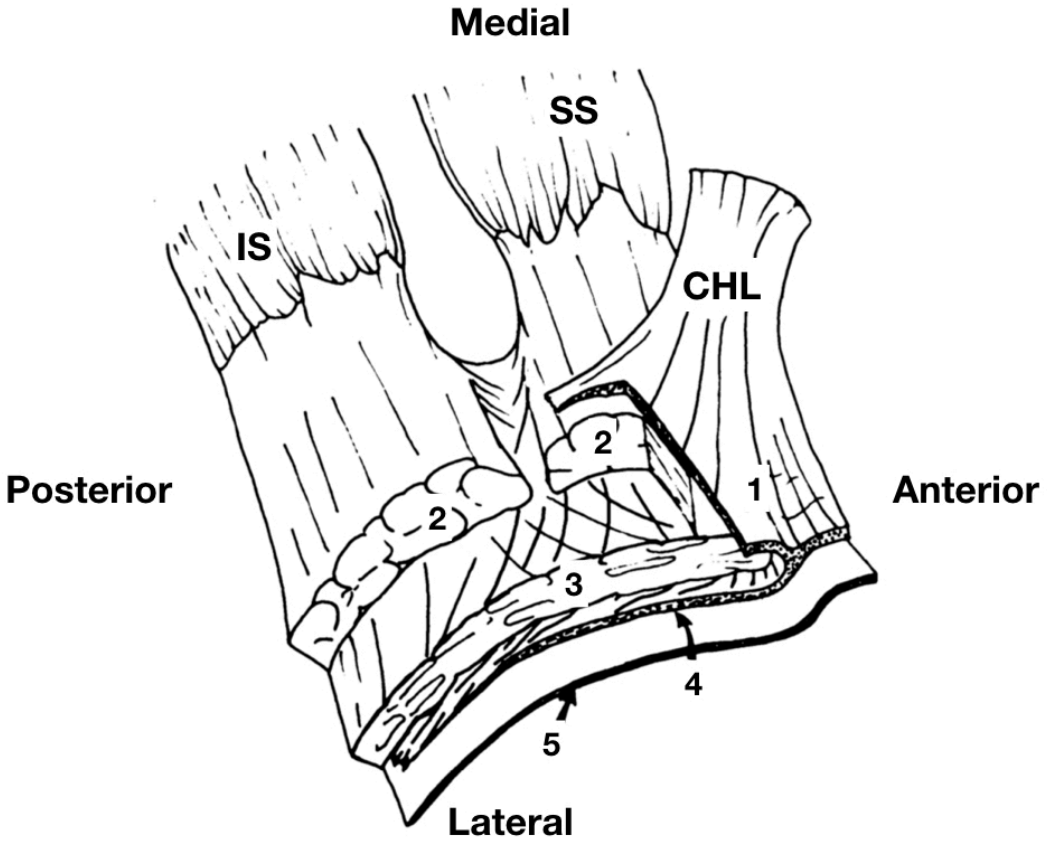


Figure 1-4: Layers of the human rotator cuff. Illustration adapted from Clark and Harryman (1992) showing the 5 layers of the rotator cuff tendon based on their histological analysis of human cadaveric samples. SS = supraspinatus, IS = Infraspinatus, and CHL = coracohumeral ligament.

In addition, the fourth layer described above forms a band of tissue that has been coined the “rotator cable”[4]. This thickened band is thought to reinforce the rotator cuff tendon. It is approximately 2.5x thicker than the tendon inserting into the humerus and can be observed as a distinct structure histologically, radiologically, and arthroscopically[5] [6].

1.2.2 Human enthesis

Tendons attach to bone via specialised fibres. Collectively, this zone of attachment is referred to as the enthesis. Perforating tendon fibres (named Sharpey's fibres) attaching to bones were first described in 1860[7]. The enthesis has later been described in 4 zones, including tendon fibres, fibrocartilage, mineralised fibrocartilage, and bone[8]. This complex and gradual transition is commonly affected by rotator cuff tears and is not reconstructed during surgical reattachment, leading to an alteration of the biomechanical properties at the new attachment site[9]. The mechanical properties of the normal supraspinatus enthesis has been studied in cadaveric and ex vivo specimens. The ultimate load and ultimate stress appears to be different dependent on the location of the fibres. In fresh frozen cadaver specimens, the anterior third of the supraspinatus has a significantly greater ultimate load (mean 411N) than the middle (mean 153N) and posterior third (mean 88N)[10]. In tissue biopsy samples taken from normal and torn supraspinatus tendons, the mean storage modulus (G) was significantly lower in torn tendons compared to normal tendons (17,600Pa vs 22,500Pa) indicating less mechanical integrity in the tendon samples from rotator cuff tears[11]. Additionally, the tensile stress within the supraspinatus tendon changes dependent on the position of the arm. Using a finite element analysis model, the tensile stress increases from 0.24 in 0 degrees of abduction to 1.65 in 60 degrees abduction[12]. In the repair situation, the torn tendon edge is brought and held to the bony footprint using sutures and suture anchors. This direct insertion of the tendon to the footprint may heal with a fibrotic scar. This 'neo-enthesis' may be sufficient to carry physiological loads, however abrupt

stress concentrations from this sudden transition from tendon to bone may explain recurrent failures[9].

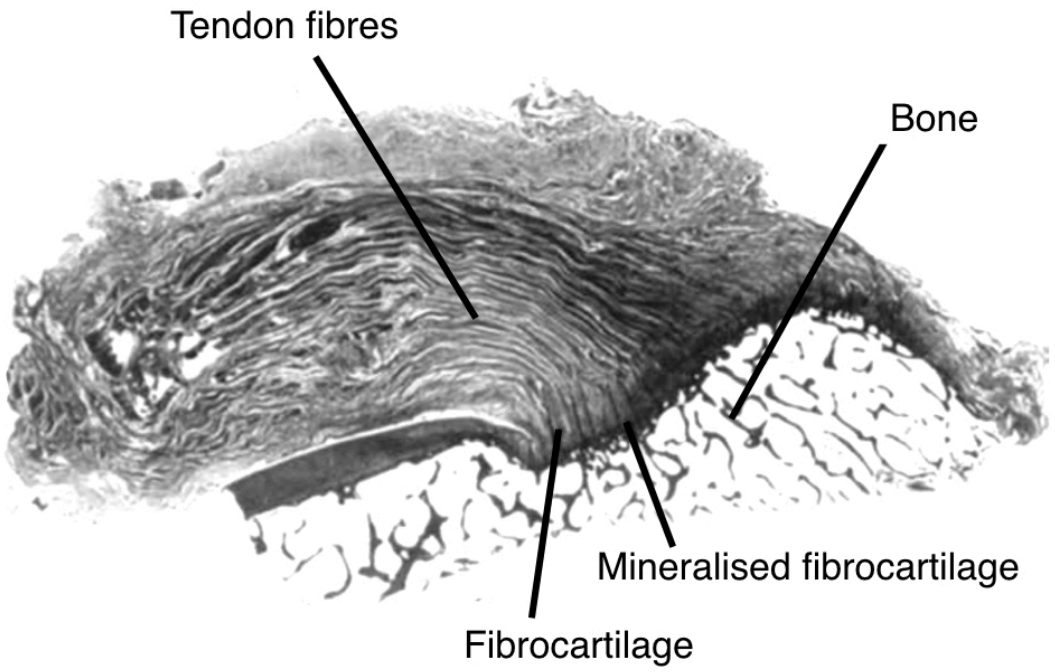


Figure 1-5: Human rotator cuff enthesis. Figure adapted from Wakabayashi et al (2003) showing the 4 zones of a normal human rotator cuff enthesis[12].

1.3 Function of the human rotator cuff

The functions of rotator cuff muscles are several. They confer significant stability to the glenohumeral joint through their action as dynamic stabilisers. Additionally, they act as a fine control muscle system, tensioning the capsular ligaments at various positions of the arm[13]. In the mid ranges of motion, the capsular ligaments are lax and therefore the rotator cuff muscles act to centralise the humeral head on the glenoid fossa, providing concavity compression. This process occurs in a proprioceptive feedback loop, allowing the cuff muscles to work as a unit to ensure the head is centralised into the

glenoid. This action allows the deltoid muscle to work efficiently, providing the majority of power in abduction. Earlier work on the biomechanics of the rotator cuff described these muscles as important for shoulder motion[14]. Supraspinatus is thought to initiate humeral abduction. Infraspinatus generates 60% of external rotation force, and resists posterior/superior translation. Subscapularis contributes to internal rotation and resists anterior/superior translation of the humeral head[15].

Force couples are seen in the rotator cuff. Force couples are two forces acting on a body, usually in opposition. This is mainly between the anterior cuff (subscapularis) and the posterior cuff (infraspinatus / teres minor), which have well-matched cross-sectional areas[16]. In addition, the supraspinatus is thought to act in a force couple with deltoid[14]. As the deltoid pulls the humerus superiorly in 0^{o} abduction, the rotator cuff acts to maintain the humeral head in the centre of the glenoid fossa[14]. In painful supraspinatus tears, the action of deltoid is diminished[17]. However, patients shoulder motion in the presence of a rotator cuff tear is not homogenous. Some patients become pseudoparalytic, unable to elevate the arm over 90^{o} abduction, whilst others may have a full range of motion with even large tears[18]. Some patients are able to compensate for reduced glenohumeral range by increasing their scapulothoracic motion[19]. Additionally, some patients with rotator cuff tears will demonstrate superior translation of the humeral head. This may be static or dynamic[20].

1.4 Rotator cuff tendon tears

Rotator cuff tendon tears are common in humans. They are found in around 15-20% of 60 year olds, 26-30% of 70 year olds, and 36-50% of 80 year olds[21 22]. A significant proportion of tears are asymptomatic[23]. Rotator cuff tendon tears cause significant pain and loss of function in some people. Patients failing conservative treatment, such as physiotherapy, analgesia, and corticosteroid injections, may proceed to surgical repair[24]. This section will focus on the background of rotator cuff tears, their assessment, clinical features, proposed aetiology, natural history, and treatment.

The term “rotator cuff tear” may relate to any of the four tendons of the cuff, however it commonly implies the supraspinatus tendon, either in isolation or as part of a larger tear that involves the other tendons. Clinically, the commonest tendon involved is supraspinatus[25]. Occasionally, isolated subscapularis tears are identified however, these are less common[26]. Rotator cuff tears can be associated with a traumatic event. Patients will often report normal shoulder function preceding an incident, followed by pain and range of motion restriction after trauma. The nature of the traumatic event can vary from significant, such as the case in an anterior shoulder dislocation, to minor, without a significant fall or force. A significant proportion of rotator cuff tears present without a history of trauma. Some clinicians use an acute vs chronic, or traumatic vs atraumatic classification to describe rotator cuff tears. However, given the high rate of asymptomatic tears in the general population, it is not possible to know whether

acute minor trauma contributes to the initiation of a tear, or whether this event coincided with a conversion from an asymptomatic tear to a symptomatic one.

1.4.1 Rotator cuff tear assessment

One way to categorise rotator cuff tears is by tear pattern. Since the popularisation of shoulder arthroscopy, the pattern of rotator cuff tears can be better appreciated. In the context of rotator cuff tears, associated tear patterns and concomitant pathologies have been described[27]. Common tear patterns include crescent, L-shaped, reverse L-shaped, U-shaped, and massive retracted. These often relate to postero-superior tears. Some tears of supraspinatus will not involve infraspinatus and the posterior cuff, but rather be associated with a tear of the upper third of subscapularis[28].

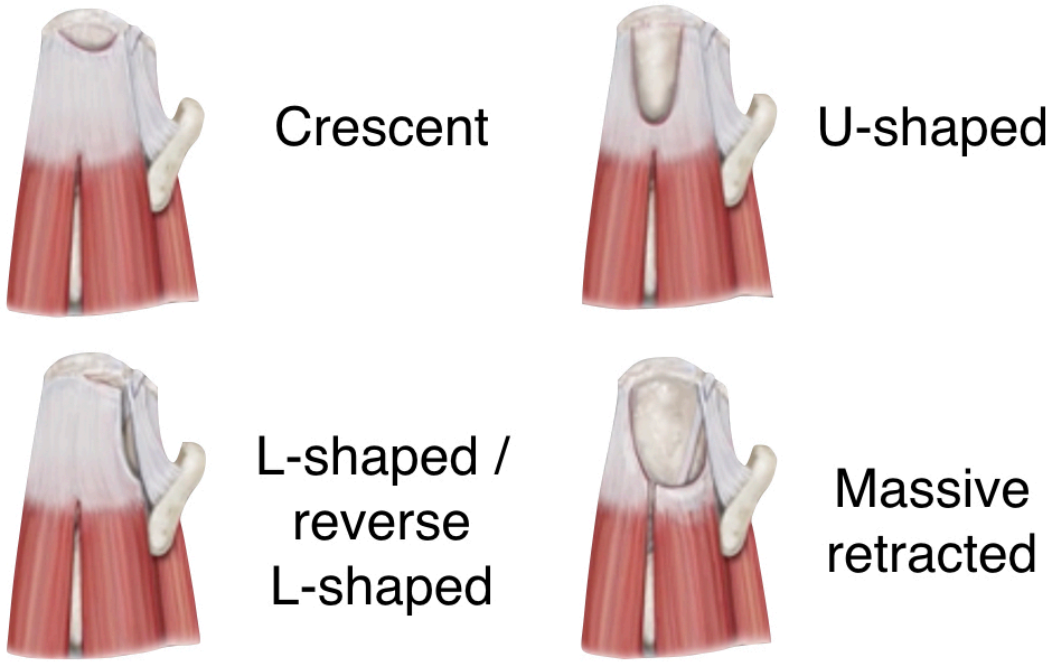


Figure 1-6: Rotator cuff tear patterns as described by Peter Millett showing common rotator cuff tear patterns of posterosuperior cuff tears[27].

Crescent tears account for approximately 40% of rotator cuff tears[27]. L-shaped and reverse L-shaped tears, which can often be difficult to identify, account for around 30% of cuff tears[27]. U-shaped tears are seen in 15% of surgical cases[27]. Massive retracted cuff tears account for less than 5% of tears. The surgical management of rotator cuff tears will require accurate identification of these different tear patterns. Each tear will behave differently, and requires identifying the degree of mobility, level of retraction, and appreciation of how to restore anatomical reconstruction[27]. A number of surgical techniques have been proposed for each tear pattern including marginal convergence, and interval slides[29 30].

Aside from chronicity of symptoms, presence of preceding traumatic event, and tear geometry, a simple classification describes the proportion and number of tendons involved. Single tendon tears (of supraspinatus) may involve part or all of the supraspinatus footprint. Two tendon tears (usually supraspinatus and infraspinatus) involve 100% of supraspinatus and <100% of infraspinatus. Tears involving more than 2 tendons are often called “massive” tears, and will often involve all of supraspinatus, all of infraspinatus, and some involvement of subscapularis. There are many other classification systems described for describing rotator cuff tear size[25]. Some utilise magnetic resonance imaging (MRI) or ultrasound scan (USS) measurements, however the reliability of these is questionable. Others use measuring probes at the time of arthroscopy, however not all repairs are performed arthroscopically. Tear size measurements

can be either in the anterior-posterior dimension, or the medial-lateral dimension. Some authors have used both to describe the size of cuff tear[31-33].

1.4.2 Symptoms of rotator cuff tear

Symptomatic rotator cuff tears will often feature pain, some degree of weakness, and some reduction in range of motion. Patients with a rotator cuff tear and pain will often describe the pain being worse at night, affecting sleep quality, and diffusely centred around the lateral aspect of the upper humerus. Weakness is often a feature of rotator cuff tears. Clinical examination may demonstrate weakness against resistance however, it is often difficult to distinguish true physiological weakness from weakness due to pain inhibition. Pain localisation is often a poor clinical sign for identifying tears of different sizes and of various tendon involvement. However, weakness in a particular motion can help identify which tendons may be involved. Surgeons have attempted to record strength using dynamometers with some reliability using handheld devices[34]. Reduced range of motion, particularly in abduction is often a feature. Patients with infraspinatus tears may have a deficiency in external rotation and patients with subscapularis tears may have reduced internal rotation. A clinician may be able to elucidate a lag sign by placing the patient in maximal external rotation and then letting go. A tear involving infraspinatus will be evident as the patient would be unable to maintain this maximal external rotation position[35]. A similar lag sign can be demonstrated in subscapularis tears.

Following a thorough history and clinical examination, a clinician will be able to identify a significant proportion of rotator cuff tears. To confirm diagnosis, further radiological investigations may be performed. In the UK, the choice of investigation for suspected rotator cuff tears is either magnetic resonance imaging (MRI) or Ultrasound (USS). These have been shown to have adequate sensitivity and specificity for full thickness rotator cuff tears[36]. Other investigations that may be required are radiographs, which may demonstrate superior migration of the humeral head in larger tears, or rotator cuff arthropathy seen in late stage disease.

1.4.3 Theories of rotator cuff tear aetiology

How cuff tears initiate is not well understood. There are several theories as to why humans are prone to this condition (Table 1-1). One key observation is that cuff tears rarely present in people before their 5th decade of life[22]. This has led to a propagation of the belief that rotator cuff tears are an "age-related degeneration" phenomenon. 'Degeneration' is a particularly unhelpful term as it labels a pathomechanism that is poorly understood, giving the impression that it is well defined. Additionally, there are some people in the 9th and 10th decades of life with an intact rotator cuff, which goes against the idea that all rotator cuff tendons will eventually fail.

Another theory is that of a vascular cause. Researchers have observed that most small rotator cuff tears are located approximately 1cm posterior from the anterior insertion of supraspinatus[37]. Combined with an anatomical studies of

vascularity in the rotator cuff demonstrating a watershed area at this location, with significantly reduced vascularity[38 39]. Some have proposed that the common location of tears seen at the watershed area are an indication of a vascular cause in rotator cuff tear initiation[40]. However, our group has identified an increase in blood vessels in patients with small cuff tears on histology[41].

Another popular theory is that of extrinsic compression causing tear initiation. Charles Neer described impingement lesions on the rotator cuff and propagated the theory that acromion morphology causes cuff tears, based on his experience[42]. Consequently three types of acromion morphology were described[43]. It was proposed that a particularly hooked acromion will compress the supraspinatus tendon during abduction of the humerus, leading to repetitive compression and tear initiation. This theory was largely born out of the observation that different acromial shapes exist. It is intuitive to consider that the underlying supraspinatus is damaged by repetitive attrition on the underside of the acromion. However, this theory was not tested objectively in the early stages. Supported by many observational studies correlating acromion morphology with cuff tears requiring surgery, this theory gained significant traction. It proposes a mechanical cause from an imperfect shoulder anatomical design leading to the high rates of cuff tears seen. More recently, Gerber et al described a new method of assessed acromion coverage over the cuff tendon, the critical shoulder angle[44]. Again, this new technique of identifying acromion morphology – cuff tear incidence correlation has been supported by level IV observational studies[45]. Theories that propose to explain a pathological condition

or observation are a necessary step in the discovery of possible treatments. However, theories that are never tested objectively, without mitigating for various prejudices, may introduce bias. Bias in experimental studies may lead to hypothesis being incorrectly accepted. When Neer, in 1983 reported that he "believed" that 95% of cuff tears are caused by "impingement" on the underside of the acromion, an appropriate next step would have been to design an experiment or series of experiments that test this hypothesis. However, instead, he propagated that decompression surgery was a necessity in conjunction with cuff repair. There are several factors that likely played a role to this theory being pervasive in the Orthopaedic community without objective, unbiased, evidence to support it. Not only is this explanation intuitive and easy to grasp, the intervention of decompression surgery is relatively easy to perform. Decades later, although still relatively infrequently undertaken, randomised controlled trials in Orthopaedic surgery have thankfully become the gold standard for determining clinical effectiveness. Several randomised controlled trials comparing healing rate and functional outcomes of patients undergoing rotator cuff repair with and without acromioplasty have shown no benefit of removing the part of the acromion thought to be causing cuff tears[46 47]. This theory does not however fully explain why some patients with a hooked acromion do not experience cuff tears. Additionally, if cuff tears are caused by the underside of the acromion, it is difficult to explain the presence of intra-tendinous or articular-sided partial thickness rotator cuff tears.

An intrinsic theory has also been proposed. This intrinsic theory has been borne out of the observation that tissue samples of rotator cuff tears demonstrate immune cell populations and proinflammatory mediators[48 49]. It is postulated that recurrent subclinical inflammation and fibrosis occurs prior to tear initiation. Hence, even small rotator cuff tears are in fact late stage disease with tendinopathic changes[50]. Whilst this theory is supported by immunohistochemistry analysis of torn rotator cuff tendon tissue samples, the pathomechanisms leading to these observations remain unclear. This theory may also explain the high recurrence rates following surgical repair of cuff tears, described later in this chapter. During surgery, the torn tendon is reattached to the bony footprint from which it has torn. However, surgery in itself does not reverse the intrinsic processes that may have caused the tear. Therefore, recurrence is likely in a significant proportion of patients.

Table 1-1: Extrinsic versus intrinsic theories of rotator cuff tear initiation highlighting key features of both proposed theories. PTT = partial thickness tear; FTT = full-thickness tear.

Extrinsic Theory	Intrinsic Theory
Acromion morphology causes cuff tears	Cuff tears are preceded by subclinical inflammation and fibrosis
External compression on tendon explains pain and tear initiation	Even small tears represent late stage disease
Explains progression from PTT to FTT	Cellular pathomechanisms may explain tear initiation

1.4.4 Natural history of rotator cuff tendon tears

The natural history of rotator cuff tears is not fully understood. To date, there has not been a longitudinal study following asymptomatic rotator cuff tears in a normal population. However, there have been several studies in various populations that have provided some insights into the natural history of cuff tears. Most studies either enrol the contralateral shoulder found to have an asymptomatic rotator cuff tear in those being offered surgery for a symptomatic rotator cuff tear on the other side[51-53]. Other studies have followed up patients with a symptomatic rotator cuff tear who declined surgery to report on their symptoms, tear size, and secondary changes[54-57]. Finally, some studies have followed up partial thickness tears, attempting to identify risk of progression to

full thickness tears[58]. From these studies we can gain insight into the following: 1) the proportion of asymptomatic tears that become symptomatic over time. 2) the proportion of tears progressing in size and demonstrating secondary muscle changes. 3) the rate of progression from partial thickness to full thickness tears.

Patients founds to have an asymptomatic rotator cuff tear on the contralateral shoulder during bilateral ultrasound investigations for a symptomatic cuff tear were followed up for 3-8 years[52]. Of the 45 patients in this retrospective study, 23 become symptomatic at an average time of 2.8 years. The authors noted a difference in mean age in those in the asymptomatic group (72.4, range 58-85) compared to the symptomatic group (67.3, range 52-82)[52]. In a larger study by the same group, they noted 44 of 195 asymptomatic patients became symptomatic at a mean of 1.93 years (range 0.4 - 4.4 years)[53]. They also noted that those patients that developed pain during the study period had larger tears at baseline and a greater increase in tear size on repeat ultrasound imaging[53]. Both study cohorts included a population of patients that have a symptomatic tear on the contralateral side, hence we cannot necessarily extrapolate these findings to patients with an incidentally-identified unilateral asymptomatic tear. The reasons and mechanisms as to why some patients become symptomatic are not clear. However, most authors in this field suggest that tear size and tear progression play a role.

A retrospective study of 24 patients under the age of 65 years who declined surgery were followed up with repeat imaging between 27-87 months after

diagnosis[54]. This study concluded that isolated supraspinatus tears did not progress over time and fatty infiltration of the muscle, as assessed by Goutallier grade on computed tomography, did not increase[54]. In another study of 71 patients (80 shoulders) with symptomatic full thickness cuff tears that declined surgery, showed on MRI at 1 year that the mean progression in tear size was 3 ± 3mm[57]. They reported that in 16 of 80 shoulders, the tear size increased by more than 5mm in 1 year. 31 of 80 tears became asymptomatic. They also concluded that tears measured between 1-2cm in the medial-lateral dimension were more likely to progress at 1 year than smaller tears (<1cm) and larger tears (>4cm), although they did not identify the mechanism for this[57]. In a similar retrospective study of 61 tears in 51 patients under the age of 60 with a full thickness tear greater than 5mm treated non-surgically, the authors reported at follow-up ultrasound (mean 29 months, range 25-39 months), mean tear size increased from 12 ± 7mm (range 5-39mm) to 19 ± 10mm (range 5-50mm)[55]. They reported that half of patients demonstrated an increase in tear size and that persistent pain at follow-up imaging correlated with an increase in tear size[55]. Another retrospective study of 174 symptomatic cuff tears treated conservatively reported the change in tear size on MRI[56]. Repeat MRI was performed at a mean of 19 months (range 12-60 months) and showed 82 of 174 tears increased in size. They reported that medium tears and being a smoker correlated with tears that increased in size[56].

In the last study, it was noted that full thickness tears progressed more often than partial thickness tears, with 34 of 83 partial thickness tears progressing in

size[56]. Kong et al reported the natural history of 81 high grade (>50% tendon thickness) partial thickness cuff tears using MRI at a mean of 20 months (range 12-52 months)[58]. They demonstrated that 13 of 81 partial thickness tears progressed, and some tears (20 of 81) "healed" or reduced in size[58]. All studies reporting the natural history are biased, retrospective, and lacking in standardised methodology. Therefore, whilst these studies have shown 1) around 25-50% of asymptomatic tears become symptomatic, 2) small and partial thickness tears progress to larger tears infrequently, 3) medium sized tears of supraspinatus probably increase in size and become symptomatic more often than other tear sizes, these findings should be taken with caution.

1.5 Rotator cuff repair

Rotator cuff repair involves securing the free tendon edge to the anatomical bony footprint on the humeral head from where it has detached. Rotator cuff repair incidence has increased in the United States to over 98 per 100,000 people[59] in 2006, with over 270,000 being performed per annum[60]. Around 17,000 rotator cuff repairs are performed in the National Health Service (NHS) in the UK each year[61]. The first description of rotator cuff tear was made by Alexander Monro in 1788[62]. Codman however, in his 1934 book The Shoulder, discusses his attempted repair, in 1911, of 2 patients with rotator cuff tears using heavy silk sutures[63]. Early descriptions of rotator cuff repair used sutures passed through the tendon edge and then through the greater tuberosity via bone tunnels[64]. Shoulder arthroscopy came much later than knee arthroscopy. The first description of shoulder arthroscopy was by Michael Burman into 25

cadaveric shoulder joints in 1931[65]. However, it was not until the 1970's that surgeons attempted diagnostic and therapeutic procedures in shoulder arthroscopy. In the last 50 years, there has been a tremendous growth in shoulder arthroscopy. This has yielded numerous advancements in our understanding of pathology, surgical techniques, and implant innovation. The evolution of rotator cuff repair techniques, and accompanying innovation in implants will be discussed next.

1.5.1 Evolution of rotator cuff repair techniques

Rotator cuff repair techniques evolved as our understanding of the biomechanical principles underpinning tendon to bone reconstruction developed. With the use of laboratory-based techniques, new methods of surgical repair have been evaluated and often shown to improve the failure characteristics[66-73]. Bone tunnel fixation has been compared with suture anchors on cadaveric and animal specimens[71 72 74]. The results have often been contradictory between studies however, suture anchors have become popular, likely due to their ease of deployment[72]. Since the advent of suture anchors, placed into bone as a method of fixation for the tendon edge, surgeons have described various constructs to secure the tendon edge to the bony footprint. A simple single row of anchors may be placed. The sutures loaded into these anchors are passed through the tendon edge and secured by knots. This is referred to as a single row repair. Another technique is to place two rows of anchors into the footprint, a medial row and a lateral row. The medial row of anchors is placed at the median part of the bony footprint, close to the articular margin. Sutures from these

anchors are passed through the tendon edge, close to the musculotendinous junction. However, care must be taken not to pass these medial sutures too close to the musculotendinous junction, as this has been shown to cause early failure[75]. These sutures can be tied on top of the tendon and then passed into a lateral row of anchors to secure them to the lateral part of the footprint. This double row fixation provides additional security to the repair. If the medial row of anchors fails, the lateral row may still maintain the reconstruction. Additionally, this double row fixation has been shown to provide greater footprint compression than a single row technique[71]. A transosseous equivalent (TOE) technique has also been described[76]. Here, the fixation is similar to the double row technique, but the lateral row is placed further lateral and inferior. This has several advantages including reducing the number of anchors occupying the footprint, as the lateral row anchors are now distant. Secondly, this technique has been shown to further increase footprint compression and load to failure compared to the double row technique[77].

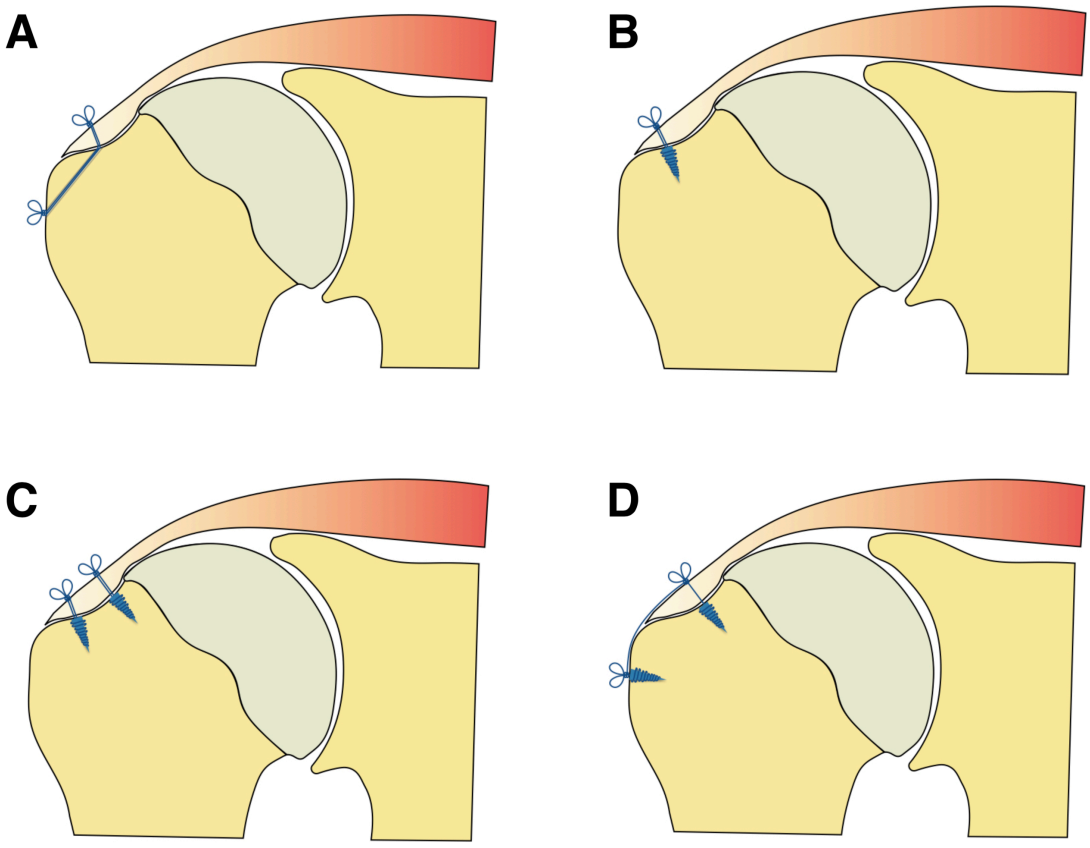


Figure 1-7: Rotator cuff repair constructs. Illustration demonstrating frontal view of different rotator cuff repair constructs including A: Transosseous repair, B: Single row repair with suture anchors, C: Double row repair with suture anchors, and D: Transosseous equivalent (TOE) repair with suture anchors.

In addition to the above techniques, there are several others that have been described to avoid various issues related to failure by suture pullout[67]. The surgical goal of a rotator cuff repair construct is to compress the tendon onto the footprint, and minimises gap formation between the tendon and bone during cyclical loading[76]. Traditionally, knots have been used to secure the sutures passed through the tendon. Some laboratory studies have tested the

biomechanical properties of knotless, knotted, and hybrid (knotted medial row and knotless lateral row) constructs[72 78]. It appears that knotless repairs undergo slippage at lower loads and fewer cycles of loading than knotted repairs[79]. Knotless repairs are undoubtedly easier to deploy for surgeons as it requires less reliance on the surgeon's knot security. Currently, surgeons preference dictates the type of repair constructs for different tear patterns. Clinically, there are no randomised controlled trials comparing knotless and knotted repairs, however cohort studies have shown no difference in clinical outcomes[80].

1.5.2 Evolution of implants used in rotator cuff repair

Suture anchors are made by numerous companies today. They are generally categorised by size, material, and deployment mechanism. Most anchors come in a variety of lengths and widths allowing versatility in their application. Early anchors were made from stainless steel. Newer anchors tend to be made from titanium, PEEK, or biocomposite. Anchors can be deployed using a pointed tap that is inserted to a particular pre-determined depth, or by drilling a hole to the corresponding dimensions. Some anchors come with a self-tapping feature, which allows for one step deployment. Anchors maintain their position in bone by using a number of different designs. The most popular design is that of a thread similar to a screw thread around a core. The pitch and depth of thread varies between manufacturers.

Another innovation is with vented anchors, where the core has vents to allow for fluid to pass from the bone to the repair site. This has been proposed to allow growth factors exuded from the bleeding bone surface to reach the healing tendon. Additionally, these vented anchors may allow bone ingrowth and therefore better security in the humeral head, however this has not been investigated outside the manufacturer's white paper pilot study[81]. These vented anchors have not been shown to improve healing rate.

Recently, all-suture anchors have been developed. These are anchors made entirely from suture material that has been designed to change shape on deployment into the bone, thereby securing them. These all-suture anchors generally occupy less bone in the footprint at the expense of less pull-out strength compared to PEEK or metal anchors[82]. The options available to surgeons today for suture implant choice are massive and largely determined by surgeon's preference. There are numerous companies producing large numbers of variations in suture anchors for every surgeon preference

Each company produces anchors preloaded with a variety of suture options, all of whom are strong, multifilament, braided, synthetic, non-absorbable sutures of varying sizes. Recently, the innovation of suture tape has been developed. Suture tape is a multifilament, braided, synthetic, non-absorbable suture with a profile designed to improve compression of the tendon to the underlying bone (Figure 1-8). These suture tapes have been tested in the laboratory setting and demonstrated less tendon elongation on cyclical loading, higher load to failure, and greater stiffness compared to multifilament braided sutures when applied to bovine rotator cuff tendons[83]. In another study using cadaveric rotator cuff tendons, they showed better footprint coverage, less displacement, and greater ultimate load to failure however, the tape was used in a linked double row construct while the suture comparator was used in a single row construct[84]. However, another study showed higher cutting rates with FiberTape suture through cadaveric rotator cuff tendon compared to FiberWire suture[85]. Similar with vented anchors, these tape suture have not been shown to improve clinical outcomes.

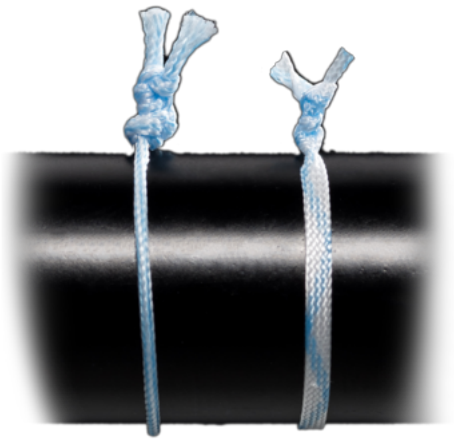


Figure 1-8: Synthetic multifilament braided suture and suture tape used in rotator cuff repair. Photograph of FiberWire suture (left), and FiberTape suture (right) (Arthrex Inc., Naples, USA) demonstrating different profiles[86]. Note the wider profile and greater surface area of the tape suture compared to the original suture.

1.5.3 Assessing outcome following rotator cuff repair

The outcome of rotator cuff repair can be determined in a multitude of ways.. Outcome assessment can be broadly categorised into: Patient Reported Outcome Measures (PROMs), radiological evaluation, and clinician-administered outcome scores. PROMs are an important part of assessing the effectiveness of medical interventions. They allow patients to report their symptoms and function in a standardised manner that can be compared longitudinally to show changes over time. Additionally, the measurable factors that surgeons may traditionally record are not necessarily aligned with what patients feel are important to them. As such, numerous patient reported outcome measures have been proposed. Unfortunately, all of these have their limitations. Some of the older scores were

developed by clinicians without any input from patients. Others have never been validated for construct validity, reliability, or responsiveness[87]. The Oxford Shoulder Score is the most valid and reliable measure for assessing patients with symptoms related to their rotator cuff[88 89].

Table 1-2: Commonly used patient reported outcome measures for shoulder pathology related to the rotator cuff. Scales, format, and presence of validation are outlined for each measure. *Note that the Oxford shoulder score was initially developed as a 12 - 60 point score (items scored 1-5), which was reconfigured to a 0 – 48 scale (items scored 0 – 4)[89].

Name	Scale	Format
Oxford Shoulder Score (OSS)*	0 - 48	12 items scored 0 – 4 (4 items related to pain, 8 items related to function)
American Shoulder and Elbow Society (ASES)	0 - 100	11 items (1 pain item, 10 function items), with 0-3 points per item. Score calculated by multiplying pain score (max 10) by 5 added to total function score (max 30) multiplied by 5/3.
UCLA Shoulder Score	0 - 35	1 item per domain in 5 domains (pain 10, function 10, active forward flexion 5, strength of forward flexion 5, and overall satisfaction 5)

Constant-Murley Score	0 - 100	35 points for subjective assessment (1 item for pain, 4 for function), 65 points for physical examination (items for range of motion and force resistance in abduction)
Western Ontario Rotator Cuff Index (WORC)	0 - 2100 converted to 100 - 0%	21 items (6 items for symptoms, 4 item for sport/recreation, 4 items for work, 4 items for lifestyle function, and 4 items for emotional wellbeing)
Rotator Cuff Quality of Life (RC-QoL)	0 – 3400 converted into a 0 - 100%	34 items in 5 domains (symptoms: 16 items, sport/recreation: 4 items, work-related concerns: 4 items, lifestyles issues: 5 items, and social/emotional issues: 5 items) recorded 0 - 100mm
Simple Shoulder Test (SST)	N/A	12 yes/no questions covering comfort, function, sleep, and employment
Shoulder Pain and Disability Index (SPADI)	0 - 100	13 items (5 pain items, 8 disability items). Calculate average score (0-100) per domain

Disabilities of the Arm, Shoulder, and Hand (DASH)	0 - 100	30 items assessing level of disability in various activities

Another method of assessing patients with shoulder pathology is by testing strength. The Constant-Murley score included in Table 1-2 is strictly a clinician-administered score that includes a strength component. There are several other ways in which strength may be recorded however, one method using a handheld dynamometer has been validated for use[34]. In reality, it is unclear whether reduced strength as assessed by any method is a result of a structural defect or from pain caused by the pathology.

Patients are now able to report their subjective assessment of shoulder pain and function via a number of outcome measures described above. Clinicians are also able to systematically record their examination findings, supplementing these with strength testing using a dynamometer if required. The surgical goal of rotator cuff repair is to restore the tendon to bone interface. However, a holistic approach would be to only consider whether the patient's symptoms resolve and their expectations of function are matched after surgery. The UKUFF trial, the largest randomised controlled trial to date involving rotator cuff repair surgery demonstrated that 85% of patients' symptoms and function improved after 2 years, despite a lower rate of healing (60% overall) assessed at 12 months[24]. This discrepancy is seldom noted by clinicians as scans to determine the healing status of the rotator cuff repair are not routine. Therefore, we must consider whether an intact repair is clinically superior to one that has failed to heal despite symptom improvement.

First however, we must consider how healing following rotator cuff repair is assessed. Clinical examination and patient reported scores are inaccurate at determining cuff repair integrity[90]. There are several imaging modalities that have been used to assess healing in the published scientific literature. Magnetic resonance imaging has become a popular method for diagnosing rotator cuff tears, and for assessing healing after surgical repair. One classification that has become popular, divides the findings of the repair on a T2-weighted MRI image as:

- Type I: repaired cuff with sufficient thickness compared to a normal cuff, and with homogenously low intensity signal.
- Type II: repaired cuff with sufficient thickness compared to a normal cuff, and with partial high intensity signal.
- Type III: repaired cuff with insufficient thickness (<50% compared to a normal cuff thickness), and without discontinuity.
- Type IV: presence of minor discontinuity observed in >2 slices in both coronal, and sagittal image planes.
- Type V: presence of major discontinuity in >2 slices in both coronal and sagittal planes.

Sugaya et al suggested that Type IV correlated to a small full thickness recurrent tear, and Type V to a medium or large full thickness recurrent tear[91]. Other groups have assessed the inter- and intra-observer reliability of this classification as fair to moderate[92]. Reducing this classification to two categories improved the reliability[92].

Ultrasound evaluation of the repaired rotator cuff tendons can be made to assess repair integrity. This technique is dependent on the assessor. Whilst the advantage is that it is a dynamic investigation, it is disadvantaged by the inability to review the images accurately at a later time (although static images can be saved). There are no conclusive studies investigating the reliability and validity of ultrasound imaging in the postoperative rotator cuff, however a recent pilot study suggested it may be reasonable[93].

Computed tomographic arthrography has been used to assess healing following cuff repair. The benefit of this method is that the contrast media can give an indication of a watertight, or completely intact repair. However, the need for ionising radiation, and intra-articular contrast are disadvantages. This technique has not been tested for its reliability and validity for detecting repair defects. It has been used in two studies to assess structural outcome of the repair[94 95], and in 3 further studies in combination with magnetic resonance arthrography (MRA)[96-98]. There is a debate about whether repair integrity influences functional outcome. Some have suggested that healing does not influence clinical outcome[99 100], whilst others have demonstrated improved outcomes in healed repairs compared to non-healed repairs[101 102]. The UKUFF trial reported a clinically significant improvement in Oxford Shoulder Score in patients with an intact repair at 1 year, than those with a failed repair[24].

1.5.4 Healing rate following rotator cuff repair

Healing rate following rotator cuff repair is well reported, mainly in retrospective observational studies. Most studies have a high risk of bias, both in their methodological design, and quality of reporting. Additionally, there is no consensus on how best to record tear size. There is also no gold standard for determining a healed repair versus a non-healed repair. There is significant heterogeneity in how studies report healing using a variety of different radiological modalities at a variety of time points. Additionally, most studies are prone to significant risk of bias, usually related to the methodological design. Hence, the range of reported healing following cuff repair in the published scientific literature is 6 - 94%[103 104]. Forty-nine studies have reported an overall healing rate of 67.8% in patients with average age of 59.6 years. Of these, 21 studies were randomised controlled trials (RCTs), 1 pilot RCT[105], 6 Quasi-randomised Controlled Trial[106-111], 6 prospective cohort studies[112-117], 4 retrospective cohort studies[118-121], 5 case-control studies[33 119 122-124], 2 prospective case series[103 125], and 4 retrospective case series[104 126-128]. Of the 28 studies reported as Level 1 evidence, there is still significant risk of bias. Table 1-3 shows an assessment of the risk of bias in the RCTs that have reported radiological assessment of cuff repair integrity as an outcome, as determined by the Cochrane Risk of Bias Assessment Tool[129].

Table 1-3: Risk of bias assessment of Randomised Controlled Trials. Trials reporting healing rate were assessed using Cochrane Risk of Bias Tool (Y = yes; N = no; U = unclear).

Year	1st Author	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?	Free of other bias?	Journal impact factor	Registered with trial register?
2011	Castricini [130]	Y	Y	Y	Y	Y	U	3.792	Yes
2013	Milano [131]	Y	U	Y	Y	U	Y	3.191	No
2012	Ma [107]	Y	Y	N	U	Y	Y	3.103	No
2013	Antuna [105]	Y	U	Y	Y	Y	N	0.567	Yes
2014	Malavolta [132]	Y	Y	Y	N	Y	N	4.362	Yes
2007	Franceschi [133]	Y	Y	N	Y	Y	N	3.397	No
2011	Koh [134]	Y	N	Y	N	Y	Y	3.024	No
2015	Kukkonen [135]	U	Y	Y	Y	U	U	5.28	Yes
2012	Lapner [32]	Y	Y	N	U	Y	U	3.234	No
2010	Moosmayer [136]	Y	U	N	Y	Y	U	2.351	No
2013	Moneo [137]	N	U	Y	U	Y	Y	3.191	No
2014	Keener [138]	Y	U	Y	N	Y	N	5.280	Yes
2009	Burks [139]	Y	U	N	Y	Y	N	3.605	No

2013	Jo [31]	Y	U	y	U	N	U	4.699	Yes
2014	Zumstein [140]	U	U	Y	Y	N	U	2.289	No
2012	Gumina [141]	U	N	Y	U	Y	U	3.234	Yes
2011	Randelli [142]	U	Y	Y	U	N	N	2.747	No
2015	Jo [143]	Y	U	N	N	Y	N	4.362	Yes
2012	Cuff [109]	N	U	N	Y	Y	N	2.319	No
2013	Gartsman [110]	Y	N	N	Y	N	N	2.365	No
2012	Carbonel [144]	U	U	N	Y	U	U	2.319	No
2014	Moosmayer [145]	U	Y	N	U	N	U	5.280	Yes
2012	Barber [106]	U	U	Y	N	N	U	3.103	No
2012	Kim [146]	U	U	N	U	U	U	4.439	Yes
2012	Lee [108]	U	U	U	N	N	U	3.103	No
2012	Rodeo [147]	U	U	U	N	N	N	4.439	Yes
2012	Arndt [111]	U	U	N	U	N	N	1.061	No
2013	Weber [148]	N	N	N	N	N	N	4.699	No

Of these 28 studies, weighting the reported healing rate by the number of patients in each study, and pooling studies by year of publication, it is noted that the healing rate reduced from 94.2% in 2007, to 70.9% in 2015 (Figure 1-9). However, it is unlikely that the healing rate actually reduced over this time, or that surgeons became worse at performing rotator cuff repair.

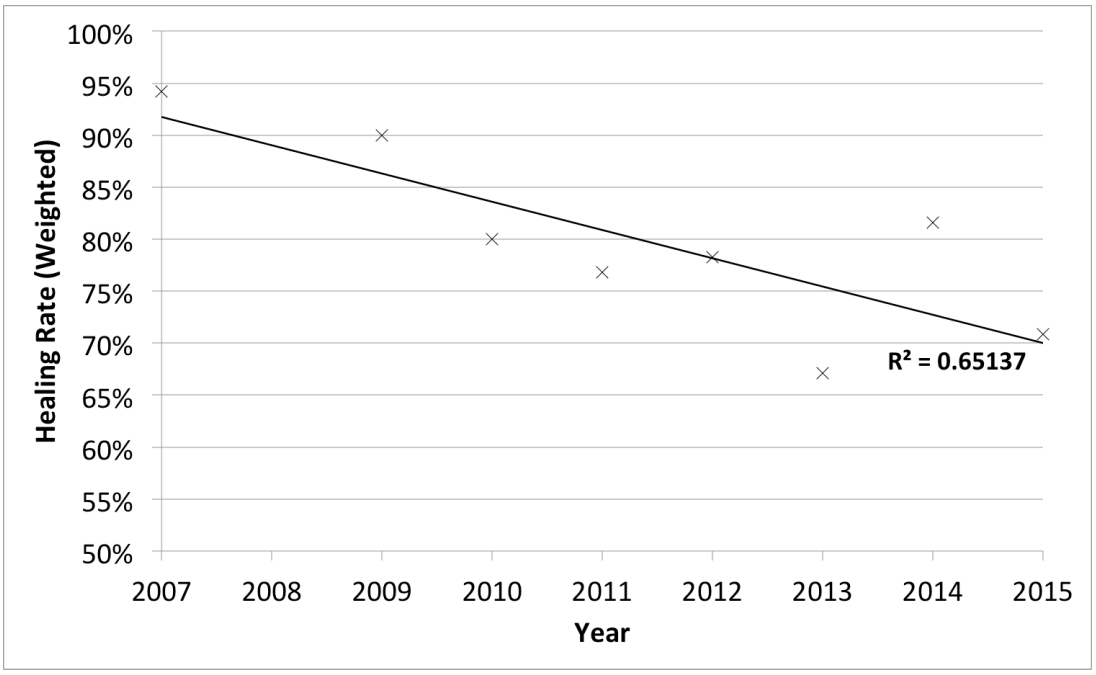


Figure 1-9: Reported rotator cuff healing in clinical trials, grouped by year of publication. Pooled weighted healing rate of rotator cuff repair as reported by level I studies from 2007 to 2015. Studies that include repair augmentation arm (patch or cell therapies) were included only where the control group (unaugmented) data can be used. Healing rate in these level I studies was determined using different radiological methods at varying time points. The commonest reported healing rate utilized MRI scanning at a minimum of 12 months post-operatively however these was significant heterogeneity.

Fourteen studies reported an influence of age and tear size on healing rate including, 3 RCTs, 1 quasi-randomised controlled trial, 3 prospective cohort studies, 4 case-control studies, 1 prospective case series, and 2 retrospective case series (Table 1-4). However, no study commented on the relationship between age and tear size.

Table 1-4: Studies reporting the influence of age and tear size on rotator cuff repair healing as assessed by imaging post-surgery.

Year	Journal	1st Author	Study Design	Mean age	Age range	n =	Time point for repair healing assessment	Healing rate	Does age influence healing?	Does tear size influence healing?
2012	JBJS Am	Lapner[32]	Randomised Controlled Trial	56.8	38-83	76	>12 months	72.4%	Y	Y
2012	JBJS Am	Gumina[141]	Randomised Controlled Trial	63	46-71	37	12 months	91.9%	Y	N/A
2012	AJSM	Rodeo[147]	Randomised Controlled Trial	57.2	N/A	31	12 weeks	80.6%	N/A	Y
2012	Arthroscopy	Ma[107]	Quasi-randomised Controlled Trial	61.2	40-80	53	>24 months (mean 33 months)	69.9%	N/A	Y
2006	JBJS Am	Iannotti[115]	Prospective cohort study	57	41-70	15	12 months	60.0%	N/A	Y

2015	Knee Surg Traumatol Arthrosc	Boyer[116]	Prospective cohort study	58.5	44-72	73	>12 months (median 13 months; range 12-17)	79.5%	N/A	Y
2014	International Orthopaedics	Hernigou[117]	Prospective cohort study	61	49-71	45	10 years	44.0%	N/A	Y
2014	Arthroscopy	Rhee[122]	Case-Control Study	66.6	N/A	238	>6 months	58.0%	Y	Y
2012	AJSM	Kim[33]	Case-Control Study	61.8	46-81	66	12-18 months	57.6%	Y	N/A
2012	AJSM	Robertson[123]	Case-Control Study	59.8	N/A	30	12 months	77.0%	Y	Y
2015	AJSM	Park[124]	Case-Control Study	59.7	39-80	339	> 1 year	86.7%	Y	Y
2006	Knee Surg Traumatol Arthrosc	Lichtenberg[125]	Prospective Case Series	60.9	46-74	53	>24 months (mean 26.4months)	75.5%	Y	Y
2011	AJSM	Chung[127]	Retrospective Case Series	59.5	39-80	272	Mean 13 months (+/- 5.1 months)	77.2%	Y	Y
2015	J Shoulder Elbow Surg	Kerr[128]	Retrospective Case Series	53	42-63	41	> 24 months (Mean 46 months)	76.0%	Y	N/A

1.6 Augmentation of rotator cuff repair

Given the low healing rate reported by some authors, clinicians have developed various techniques in an attempt to improve the healing rate. Some of these are related to the surgical repair, such as various methods of footprint preparation[131], whilst others involve additional steps to augment the repair. Rotator cuff repair augmentation can be broadly categorised into cellular augmentation, and patch augmentation. The commonest cellular-based therapy is platelet-rich plasma (PRP). This involves taking whole blood from a patient, centrifuging it in a specialised kit to remove erythrocytes, and harvesting the buffy coat, thought to be rich in growth factors, immune cells, mesenchymal stem cells, and platelets. PRP injections in various forms have been frequently studied[31 105 130 132 137 142 143 148]. Some studies have shown a benefit in healing rate[31 142]. A recent Cochrane review investigated the efficacy of PRP in musculoskeletal injuries, included a subgroup analysis for rotator cuff repair. This meta-analysis found no clinically significant difference in pain, function, and recurrent tear rate at 1 and 2 years[149]. Clinicians have little control over the factors present in the PRP fraction. Studies have shown significant variation between different kits, and between patients[150 151]. Additionally, this shotgun approach of injecting an unknown amount of various growth factors, cytokines, and cells, is unrefined and speculative.

An alternative strategy is to augment the repair with a patch graft. Patches used in rotator cuff augmentation may be synthetic, biosynthetic or biological. Patch augmentation can be applied to rotator cuff repair in several ways (Figure 1-10). A

commonly used technique is an onlay technique. This involves performing a standard cuff repair and then securing a patch graft over the repair. The patch may be secured using the suture limbs from the suture anchors, or by a separate suture to the tendon. An interposition technique has also been described[152]. This involves placing the patch between the native supraspinatus tendon, and the bony footprint. Finally, some surgeons have used patches to bridge a tendon defect. This may be done if the tendon is retracted, and after performing releases, the tendon edge does not reach the bony footprint. This technique is different from superior capsular reconstruction, which involves securing a patch onto the superior aspect of the glenoid and onto the footprint of supraspinatus[153]. All three techniques have been reported in observational studies without a reported comparator group (Table 1-5).

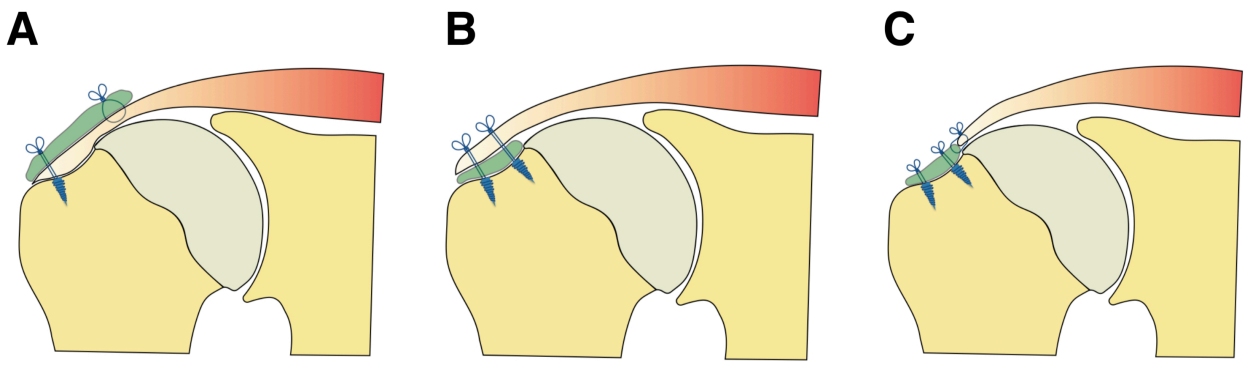


Figure 1-10: Patch augmentation constructs. Illustrations demonstrating various patch augmentation constructs described, including onlay (A), interposition or intercalary (B), and bridging (C). The patch augments are shown in green.

Biological grafts are more popular and can be broadly divided into allograft, autograft, or xenograft. Fascia lata autografts have been studied and shown reduced gapping and repair elongation in cadaveric testing[154]. Several observational studies have reported improved functional outcomes in massive tears augmented with biceps tendon autograft in small numbers of patients, with a selective healing rate of 61% on MRI from 12 months post-repair[155].

Human cadaveric dermal allografts represent the most popular patch augment used in rotator cuff repair. Several companies produce these grafts, harvested from cadavers, decellularised, and processed for human implantation. Biological patches were often initially used for other indications, such as skin defects in foot ulcers, and to augment hernia repair surgery[156 157]. Their use in rotator cuff augmentation has been predominantly in large and massive tears. Other sources of patches used in cuff repair augmentation are from porcine dermal and porcine small intestine submucosa grafts[158]. One such xenograft produced an adverse sterile inflammation response in humans, and has been withdrawn by the manufacturer[159]. Biological patches will be discussed in more detail in Chapter 3.

Finally, synthetic patches have been used to add mechanical support to the rotator cuff repair (Table 1-5). These patches are usually made from non-biodegradable polymers, and deployed to share the carrying load of the repair construct as the tendon heals[160]. Synthetic patches used in rotator cuff repair will be discussed in more detail in chapter 4.

Table 1-5: Studies utilising patch augmentation in rotator cuff repair. First author, year of publication, journal name, study design, number of patients, presence of a control group, surgical technique, and patch type are shown. RCT = randomised controlled trial. SIS = small intestine submucosa. Arch Bone Jt Surg = Archives of Bone and Joint Surgery. AJSM = American Journal of Sports Medicine. JSES = Journal of Shoulder and Elbow Surgery. JBJS Am – Journal of Bone and Joint Surgery (American Volume). Knee Surg Sports Traumatol Arthrosc = Knee Surgery, Sports Traumatology, Arthroscopy.

First Author	Year	Journal	Trial design	Number of augmented repairs	Control Group?	Technique	Patch Type	Patch name / Manufacturer
Flury[161]	2018	Arthroscopy	Cohort study	20	Yes (from previous trial who received PRP only, no patch)	Bridging	Porcine dermal xenograft	DX reinforcement matrix / Arthrex
Consigliere[162]	2017	Arch Bone Jt Surg	Prospective case series	10		Onlay	Porcine dermal xenograft	DX reinforcement matrix / Arthrex
Yoon[163]	2016	AJSM	Cohort study	21	No patch control group	Bridging	Dermal allograft	Allocover / Hans Biomed

Petri[164]	2016	Arthroscopy	Retrospective case series	13		Onlay	Dermal allograft	Arthroflex / Arthrex
Lederman[165]	2016	JSES	Prospective case series	61		Onlay	Porcine dermal xenograft	Conexa / Tornier
Bryant[166]	2016	JSES	RCT	34	No patch control group	Onlay	Porcine SIS xenograft	Restore Orthobiologic Implant / Depuy
Vitali[167]	2015	Techniques in Hand & Upper Extremity Surgery	Retrospective comparative study	60	No patch control group	Onlay after bridging with biceps autograft	Synthetic polyproplyene	Repol Angiomesh / Angiologica
Lenart[168]	2015	JSES	Retrospective case series	13		Onlay	Synthetic Poly-L-lactic acid	X-Repair / Synthasome
Gilot[114]	2015	Arthroscopy	Non-randomised, prospective, blinded, single-centre, comparative clinical study	20	No patch control group	Onlay	Dermal allograft	Arthroflex / Arthrex
Proctor[169]	2014	JSES	Retrospective case series	18		Onlay	Synthetic Poly-L-lactic acid	X-Repair / Arthrex

Ciampi[118]	2014	AJSM	Retrospective cohort-control study	49 (collagen patch) vs 52 (synthetic patch)	3 groups (inc. no patch control group)	Onlay	Bovine pericardium patch / Synthetic polyproplyene	Tutopatch (Tutogen Medical) / Repol Angiomesh (Angiologica)
Cho[170]	2014	Clinics in Orthopedic Surgery	Retrospective case series	5		Bridging	Porcine dermal xenograft	Permacol / Zimmer Biomet
Venouziou[171]	2013	American Journal of Orthopedics	Retrospective case series	14		Bridging	Dermal allograft	GraftJacket / Wright Medical
Petricciolo[172]	2013	Musculoskeletal Surgery	Retrospective case series	10		Onlay	Synthetic Polyurethaneurea	SportMesh / Biomet
Mori[173]	2013	Arthroscopy	Retrospective comparative study	24	Partial repair (no patch)	Bridging	Autograft fascia lata	
Gupta[174]	2013	AJSM	Prospective case series	27		Bridging	Porcine dermal xenograft	Conexa /Tornier

Marberry[175]	2012	Shoulder & Elbow	Retrospective case series	17		Onlay	Synthetic Polyurethaneurea / Polycaprolactone	Artelon Tissue Reinforcement / Artimplant AB
Gupta[176]	2012	AJSM	Prospective case series	24		Bridging	Dermal allograft	GraftJacket / Wright Medical
Barber[106]	2012	Arthroscopy	RCT	22	No patch control group	Onlay	Dermal allograft	GraftJacket / Wright Medical
Agrawal[177]	2012	International Journal of Shoulder Surgery	Retrospective case series	14		Onlay	Dermal allograft	Allocover / Hans Biomed
Rotini[178]	2011	Musculoskeletal Surgery	Retrospective case series	5		Onlay	Dermal allograft	Human cadaveric donors from local tissue bank
Encalada-Diaz[179]	2011	JSES	Retrospective case series	10		Onlay	Synthetic polycarbonate polyurethane	Biomerix RCR Patch / Biomerix
Wong[180]	2010	JSES	Retrospective case series	45		Bridging	Dermal allograft	GraftJacket / Wright Medical
Phipatanakul[181]	2009	American Journal of Orthopedics	Retrospective case series	11		Bridging	Porcine SIS xenograft	Restore Orthobiologic Implant / Depuy

Bond[182]	2008	Arthroscopy	Retrospective case series	16		Bridging	Dermal allograft	GraftJacket / Wright Medical
Badhe[183]	2008	JSES	Prospective case series	10		Bridging	Porcine dermal xenograft	Permacol / Zimmer Biomet
Walton[159]	2007	JBJS Am	Abandoned RCT - abandoned changed to modified outcome study with case-control	16	No patch control group	Onlay	Porcine SIS xenograft	Restore Orthobiologic Implant / Depuy
Burkhead[184]	2007	Seminars in Arthroplasty	Retrospective case series	17		Onlay	Dermal allograft	GraftJacket / Wright Medical
Iannotti[115]	2006	JBJS Am	RCT	15	No patch control group	Onlay	Porcine SIS xenograft	Restore Orthobiologic Implant / Depuy
Audenaert[185]	2006	Knee Surg Sports Traumatol Arthrosc	Retrospective case series	41		Bridging	Synthetic	Mersilene / Ethicon

Malcarney[186]	2005	AJSM	Case series - partly retrospective, partly prospective	25	Bridging	Porcine SIS xenograft	Restore Orthobiologic Implant / Depuy
Sclamberg[187]	2004	JSES	Retrospective case series	11	Bridging or Onlay over partial repair	Porcine SIS xenograft	Restore Orthobiologic Implant
Metcalf[188]	2002	Operative Techniques in Orthopaedics	Retrospective case series	12	Onlay	Porcine SIS xenograft	Restore Orthobiologic Implant / Depuy
Hirooka[189]	2002	Journal of Orthopaedic Science	Retrospective case series	28	Bridging	Synthetic Polytetrafluroethene	Gore-Tex patch

1.7 Unmet clinical needs related to rotator cuff tears

The scientific literature pertaining to rotator cuff tears is extensive, and involves a wide range of research areas. This thesis will focus on a particular aspect of rotator cuff repair, specifically healing following reconstruction of the tendon-to-bone interface. Despite many studies reporting structural integrity following repair, their remains significant uncertainty regarding the healing rate. This is mainly due to most studies being retrospective and observational in nature. Defining healing in itself is a challenge. The reliance on imaging has brought into question the accuracy and clinical relevance of these techniques. It is unknown whether repairs heal then re-tear, or fail to heal at the outset. The lack of healing is often not recognised clinically and there exists some discrepancy between symptoms, function, and structural integrity of the repair. Additionally, many researchers have identified factors thought to be prognostic for likelihood of healing. There remains dissonance in the published literature as to whether these factors influence healing, and their relative contributions to the healing rate. Therefore, there is a pressing need for clarity amongst the many observational studies with significant methodological biases. Chapter 2 will investigate the influence of age and tear size on the healing rate following rotator cuff repair. Using unbiased data from a large randomised controlled trial, the aim is to define the healing rate and the extent to which these commonly cited prognostic factors influence healing.

Despite significant innovation in surgical techniques and implants for rotator cuff repair, often demonstrating superior biomechanical properties in laboratory

studies, healing remains a problem for many patients. Surgeons have begun to appreciate that not all tears heal, and have focussed on augmenting larger tears with various strategies. Biological patch augmentation is used clinically by surgeons, despite lack of robust scientific evidence to support their use. There are no high quality, low risk of bias clinical studies utilising these implants. Their clinical efficacy is yet to be determined. Additionally, there has been no systematic prospective evaluation of the tissue response to these patches in humans. Chapter 3 will investigate the tissue response of two commercially available biological patches in augmentation of rotator cuff repair. The aim is to gain insight into the early *in vivo* tissue response to a dermal allograft and a porcine dermal xenograft.

Chapters 4 and 5 will focus on the investigation of two novel implants designed for tendon augmentation. Electrospinning technology has been used by our research group as a means of manufacturing a biodegradable polymeric composite patch and a multifilament suture. Electrospinning allows generation of nano- and microscale polymeric fibres, capable of influencing cell behaviour[190] [191]. The aim of applying this technique to tendon augmentation is to convert an inert polymer to a bioactive implant that may stimulate healing. Using strategic biomimicry, the native tendon extracellular matrix structure has been harnessed to produce these implants that have shown encouraging results in the laboratory. Chapter 4 will investigate the safety and efficacy of an electrospun polydioxanone (PDO) / polycaprolactone (PCL) patch in tendon augmentation using a validated non-healing ovine tendon injury model. The focus will be to

assess whether this implant can successfully induce tendon repair safely and without an adverse systemic or local reaction. Chapter 5 will investigate the safety and efficacy of an electrospun, twisted, multifilament, PDO suture compared to a conventional extruded monofilament PDO suture in a similar ovine tendon injury model.

Finally, chapter 6 will provide a general discussion of these experimental chapters. The key findings of this thesis will be summarised, and the clinical implications will be discussed. Chapter 6 will also discuss proposed future work in the field of rotator cuff repair augmentation. The thesis aims and hypotheses are outlined below.

1.8 Overall thesis aims and objectives

This thesis will focus on understanding and improving healing following rotator cuff repair surgery. The overall goal of this thesis is to add knowledge that may aid in improving the healing rate following rotator cuff repair. After identifying factors that influence healing, this thesis will be predominantly concerned with strategies to augment tendon repairs in order to achieve healing. The objectives of this thesis are to first better understand how the healing rate may be influenced by age and tear size. Another objective is to evaluate how biological patch augmentation influences the native tendon response. Finally, this thesis will aim to evaluate whether electrospun scaffolds can safely induce healing in tendon repair.

1.8.1 Thesis chapter aims and hypotheses

Chapter 2 will focus on investigating the influence of age and tear size on rotator cuff repair healing. The aim is to define the healing rate following rotator cuff repair using unbiased data. The hypothesis in this chapter is that the healing rate following rotator cuff repair is around 70%. An additional hypothesis is that increasing age and increasing tear size negatively influence healing rate following rotator cuff repair.

Chapter 3 will focus on investigating the early tissue response of the native supraspinatus tendon to 2 biological patches compared to a control group. The hypothesis for this work is that biological patches used to augment rotator cuff

repair cause a significant pro-inflammatory tissue response in the underlying native supraspinatus tendon.

Chapter 4 will focus on investigating the systemic, local, and cellular response of an electrospun PDO/PCL patch to augment tendon healing. The hypothesis is that an electrospun PDO/PCL patch used to augment tendon healing does not produce an adverse systemic, or local, tissue response. Additionally, another hypothesis is that this electrospun PDO/PCL patch induces tendon repair and promotes cellular infiltration.

The final experimental chapter, chapter 5, will focus on investigating the systemic, local, and cellular response of an electrospun multifilament PDO suture to repair a tendon defect. The aim of this chapter is to compare the cellular response of an electrospun multifilament PDO suture to an extruded monofilament PDO suture used in tendon repair. The hypotheses are that: electrospun multifilament PDO sutures can successfully repair a tendon defect and; these electrospun sutures promote cellular infiltration compared to an extruded monofilament PDO suture in tendon repair.

Chapter 2: The influence of age and tear size on rotator cuff healing rate

This first experimental chapter will focus on the outcome following rotator cuff surgery. The assessment and goals of rotator cuff surgery will be discussed. This chapter will report on a clinical study performing a new analysis using data from a large multicentre randomised controlled trial to determine the influence of age and tear size on healing rate following rotator cuff repair.

2.1 Outcomes of rotator cuff repair surgery

The incidence of rotator cuff repair surgery is increasing in the UK and the US[59] [192]. In order to determine the clinical impact of these operations on patients undergoing them, we must measure and record their outcomes at appropriate time points. There are many ways to assess outcome following an intervention such as rotator cuff repair[193]. Surgeons and physiotherapists often have different perspectives from patients regarding the outcomes of surgery. As a result, patient reported outcome measures (PROMS) have become a popular means of assessment. PROMS allow the patient to describe how their shoulder is functioning in a standardised and reliable manner[194]. Of the patient reported outcome measures related to shoulder pathology, many have been developed without adequate methodology, and are rarely validated appropriately for use[88]. The Oxford Shoulder score (OSS) is a measure that has been developed and validated rigorously[89] [195]. Patients are asked to answer 12 questions using a 5 point Likert scale, relating to their level of pain and ability to complete certain daily tasks in the last 4 weeks. The score is totalled, with a minimum of 0 and a

maximum of 4 points per question available, giving a possible range from 0 – 48 points. 48 points is a well-functioning and pain-free shoulder[89].

2.2 Surgical goal of rotator cuff surgery

Whilst the holistic goal to the treatment of rotator cuff tears is to return the patient to a pain-free state with a functional shoulder, the surgical goal of a rotator cuff repair is to restore the torn tendon edge to the anatomical footprint on the humeral head. This may be achieved in a variety of ways, utilising numerous different techniques, implants, and sutures[66 83 85 120 196-199]. After successful surgical repair, a period of rehabilitation is required to help restore function as the repair heals. Using validated and objective measures for assessing shoulder function after surgery, it is possible to determine the relationship between surgical factors and patient reported outcomes.

2.2.1 Does tendon healing influence patient reported outcomes?

Healing following cuff repair surgery has historically been considered as an enduring reattachment of the tendon edge to the bony footprint at a particular time point at which it is assessed. Expanding this, we can add a functional component such as, a reattached tendon to its bony footprint that is able to carry normal physiological loads, permitting full pain-free range of motion after a period of rehabilitation. The complex enthesis of the rotator cuff insertion to the humeral head, made up of various zones as the specialised Sharpey's fibres meet the bone obliquely, is one that is never restored with surgery[200 201]. Instead, a process of fibrosis and scarring leads to a repaired tendon that in most cases

is sufficient to carry out the necessary function of the enthesis[200]. It seems intuitive that a tendon that heals may provide a superior clinical outcome to those repairs that fail to heal, or have a recurrent defect. There is evidence from observational studies that have both supported and discounted the notion that a healed repair is superior to a non-healed repair[100]. However, several randomised controlled trials have reported a superior clinical outcome in healed repairs[32 202]. Recently, a large randomised controlled trial relating to rotator cuff repair surgery reported differences between healed repairs, non-healed repairs, and surgical debridement for impossible-to-repair tears[24]. The authors reported 3-4 points improvement in OSS at 2 years in those with a healed repair compared to a non-healed repair. Patients who had an attempted repair that did not heal had a further 4-7 points improvement in OSS at 2 years compared to those who had a surgical debridement without attempted repair[24].

2.2.2 Healing rate after rotator cuff repair surgery

Many observational studies have described the healing rate following rotator cuff repair. Reported healing rates range from 6% to 94%[103 104]. Interpretation of these studies is confounded by variations in the definition of healing, the time point at which healing is assessed and the imaging modality and method used to assess healing. A recent randomised trial comparing open versus arthroscopic repair (UKUFF trial) revealed that overall 40% of repairs fail within 12 months irrespective of the surgical technique used, and that a failed repair adversely affected patient outcomes[202]. Confusion also arises when considering prognostic factors that may influence healing following surgical repair. Commonly cited

factors are: age[32 122 124 146], tear size[32 107], fatty infiltration of supraspinatus muscle[203 204], muscle atrophy of supraspinatus[205], muscle-tendon retraction[127 206], gender[207], manual workers[207], workers compensation[208], poor compliance with rehabilitation[209], hypercholesterolemia, cigarette smoking[210], low bone mineral density of the humeral head [127], local corticosteroid injections, type of repair construct[110 211], and use of orthobiologics[143 147 148]. The factors that are most consistently reported are patient age and tear size. Researchers have often thought a person's capacity to heal is related to their biological age, with mechanisms for healing being different from those in utero for example, where "scarless" healing has been proposed[212]. The patient age – rotator cuff healing relationship is more of an observation and has not been definitively studied. Patients' with larger tears have long thought to have a reduced capacity for healing. Some surgeons believe that this is because it is more challenging to create a tension-free repair construct, as larger tears often have a degree of retraction, adhesion, and tendon tissue loss. Others believe that larger tears are more prone to biological changes observed as fatty infiltration and atrophy of the supraspinatus muscle, which has been correlated with lower healing rates[33 124]. One study demonstrated reduced fibroblast cellularity, reduced vascularity, and increased chondroid metaplasia in larger tears compared to smaller tears[41].

2.3 Aims

1. The primary aim of this study was to determine the influence of age and tear size, determined at surgery, on the healing rate of rotator cuff repair.

2. The secondary aim of the study was to determine if tear size and age were independent risk variables.

2.3.1 Hypotheses

1. Increasing age and tear size reduce the likelihood of healing.
2. Age and tear size are independent variables influencing healing rate.

2.4 Materials and methods

Data for this study was collected from the UKUFF trial. The UKUFF trial was funded by the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) Programme (Grant reference number 05/47/02). The UKUFF trial was a multicentre pragmatic clinical effectiveness trial investigating differences between open versus arthroscopic rotator cuff repair using the Oxford Shoulder Score (OSS) at 24 months as the primary endpoint[202]. The focus of this chapter, however, is on the influence of age and intra-operative assessment of tear size on healing, as determined by MRI imaging 12 months following surgical repair. The purpose of the study was to investigate how age and tear size influence healing irrespective of the method of surgical repair, open or arthroscopic, hence both techniques were grouped together.

2.4.1 Patients recruited for the study

The UKUFF trial included 47 recruitment centres, all of which were National Health Service (NHS) Hospitals in the United Kingdom. Patients attending an

elective outpatient orthopaedic clinic because of shoulder pain were screened. Patients recruited into the trial were over 50 years old, with a symptomatic, radiologically-confirmed, atraumatic, full-thickness rotator cuff tear. For inclusion and exclusion criteria for the UKUFF study, see Table 2-1. Of the eligible patients identified, 447 patients were randomised to open or arthroscopic rotator cuff repair in a pragmatic multi-centre parallel group randomised controlled trial, between November 2007 and February 2012[202]. The last patient follow-up was completed in December 2013. 65 surgeons in 47 hospitals performed rotator cuff repair in 256 patients. Patients underwent surgical repair (mini-open or arthroscopic) in the National Health Service (NHS). Patients who did not have a complete repair, such as those whose tear was irreparable, were not included in this study but they were followed up and reported in the main trial report. At the time of surgery, rotator cuff tear size was recorded into 1 of 4 categories (small, medium, large, and massive). Patients underwent assessment at 2 and 8 weeks by telephone and at 8, 12, and 24 months post-operatively with patient reported outcomes (the Oxford Shoulder Score [213], the Shoulder Pain and Disability Index [214], and the EuroQol-5D [215]).

Table 2-1: Inclusion and exclusion criteria for enrollment into the UKUFF trial.

Inclusion Criteria	Exclusion Criteria
Patient is aged 50 years or over	With rheumatoid arthritis
No previous surgery on affected shoulder	With severe osteoarthritis in their shoulders & other joints
Degenerative rotator cuff tear (not traumatic)	With significant neck problems
Full thickness rotator cuff tear	With dual shoulder pathology
Tear deemed suitable for surgical repair	Who have undergone previous surgery on the affected shoulder
Patient able to participate for duration of follow up	With significant cognitive impairment or language issues
Patient able to consent	Who are unable to consent for themselves
Patient summary information sheet given	Who cannot undergo an MRI scan for any reason
Patient happy to receive further information from Oxford trial office	
Patient placed on NHS waiting list	

At the 12-month post-surgical repair assessment, patients underwent evaluation of the repair with a MRI scan using a standardised protocol or, if MRI was contra-indicated, by High Definition Ultrasound performed by an experienced ultrasonographer. All scans were reviewed by a senior musculoskeletal radiologist to determine if the rotator cuff repair had healed, not healed, or if the imaging findings were inconclusive. A healed repair was defined as one where the supraspinatus tendon remained attached to the proximal humerus, whilst a failure to heal was deemed to be full thickness defect of the supraspinatus tendon. Partial thickness defects seen at the 12-month imaging scan were treated as either healed if low grade, or inconclusive if high grade. 230 patients underwent imaging at 12 months (MRI or US) of which 217 were of sufficient quality to be interpreted. 3 patients had undergone revision rotator cuff repair for a radiologically-confirmed failure of healing and were included in this analysis.

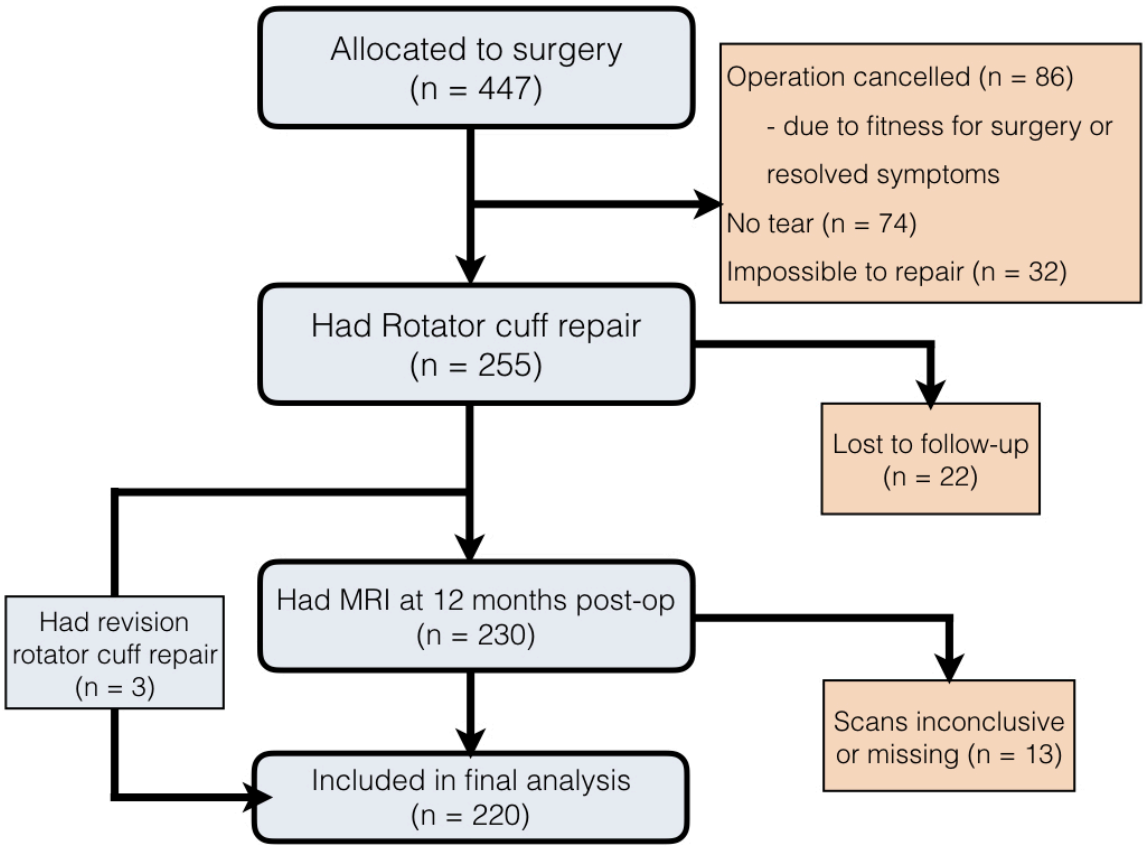


Figure 2-1: Flowchart of patients included in final analysis with full thickness rotator cuff tears treated with surgical repair in the UKUFF trial. Exclusions and reasons for these are provided in orange boxes. 220 patients were including in the final analysis, including 3 patients who underwent a revision rotator cuff repair. These 3 patients were treated as failed repairs.

2.4.2 Surgical procedure and post-operative rehabilitation

In this pragmatic clinical effectiveness trial surgeons provided usual care for their patients including the method of rotator cuff repair, type of immobilisation, and post-operative protocol for rehabilitation. Details of the surgical technique were recorded.

2.4.3 Tear size assessment

Intra-operative assessment of the tear size was used to reduce any potential error from measurements taken from pre-operative imaging and to account for any progression of the tear that might have occurred between imaging and surgery. The UKUFF trial report analysed tear size based on the assessment from pre-operative imaging. Surgeons were given education and provided with written information about how to assess tear size intra-operatively. This included schematic drawings of the shapes and sizes for each of the categories. An intra-operative data collection sheet was completed for each case. Small tears were defined as full thickness defects in the supraspinatus tendon under 1 cm in the anterior-posterior (AP) dimension. Medium tears were defined as full thickness defects in the supraspinatus tendon only, greater than 1 cm and less than 3 cm in the anterior-posterior (AP) dimension. Large tears involved full thickness defects of both the supraspinatus and infraspinatus tendons, greater than 3 cm, and less than 5 cm in the AP dimension. Massive tears involved all 3 tendons (supraspinatus, infraspinatus, and subscapularis) and were greater than 5 cm in the AP dimension. All participating surgeons met with the trial chief investigator and discussed all aspects of trial involvement including the data collection theatre form. All queries were resolved prior to commencement of recruitment.

2.4.4 Statistical analysis

Logistic regression was used to evaluate the influence of patient age and intra-operative tear size on structural integrity of rotator cuff repair at 12 months post-operatively in Stata (StataCorp, Texas, USA). Tear size was modelled into 4

groups (small, medium, large and massive). Age was initially treated as a continuous linear factor (years). Sensitivity of findings, controlling for hand dominance, previous steroid injection, sex, tear size, and age, were assessed. Age was also modelled using a fractional polynomial approach. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated.

2.5 Results

447 patients were randomised to surgery, of these, 255 underwent rotator cuff repair. 86 patients were cancelled whilst waiting for surgery because either their symptoms resolved or they had become medically unfit. At surgery, 74 were found to have no tear due to a false positive scan and in 32 the tear was impossible to repair. 3 patients underwent revision rotator cuff repair before the 12-month MRI scan time point due to radiologically-confirmed failure of the primary repair. Of the 255 patients that underwent surgical repair, 230 had a standardised assessment of the repair at 12 months post-operatively of which 13 could not be interpreted due to metal artefact from suture anchors leaving 220 patients included in the final analysis (Figure 2-1).

2.5.1 Failure rate by tear size

The overall failure to heal rate was 43% (95/220) at 12 months post-surgical repair. For small tears, the failure rate was 34% (20/58) at 12 months. For medium tears the failure rate was (27/75) 36%. Just under half, 47%, (24/51) of large tears showed an intact repair at the 12-month MRI scan, and 73% (24/33) of massive tears failed to heal.

2.5.2 Relationship between age and tear size

Patients with an intact repair were on average 3 years younger than those that did not heal (61 vs. 64 years, $p = 0.03$). For small tears, the mean age was 59 years, for medium tears 62 years, for large tears 64 years and for massive tears 66 years. To explore this relationship further, age and tear size were adjusted in the logistic regression model.

2.5.3 Influence of age on healing rate

The logistic regression model demonstrated that age was an independent predictor for a structurally intact rotator cuff repair. Following adjustment for tear size, number of corticosteroid injections, sex, and hand dominance, age remained a predictive factor for healing (OR 0.95, CI 0.91 – 0.99, $p = 0.047$).

2.5.4 Influence of tear size on healing rate

Increasing tear size was also related to a reduction in healing rate ($p < 0.001$). However, given that larger tears were observed in older patients, using a logistic regression model, age and tear size were tested to determine if both independently influenced the likelihood of a successful repair. Following adjustment for age, number of corticosteroid injections, sex, and hand dominance; only massive tears remained a predictive factor for an intact repair at 12 months (OR 0.18, CI 0.005 – 0.61, $p < 0.01$). The effect of increasing age

seen in larger tears is a confounder, that when controlled for in this model, led to only massive tear size being an independent predictor of failure to heal.

2.5.5 Estimated probability of healing

From the logistic regression model, the estimated probability of healing was determined for patients who are 50, 60, 70, and 80 years old with a small, medium, large or massive tear. In small and medium tears in 50 and 60 year olds the predicted healing rate was between 65 and 78% (Figure 2-2).

	Small	Medium	Large	Massive
50 years old	74%	78%	63%	43%
60 years old	65%	70%	52%	33%
70 years old	54%	60%	41%	24%
80 years old	43%	49%	31%	17%

Figure 2-2: Model for predicted rotator cuff healing rate at 1 year. Heat map illustrating predicted healing for patients aged 50, 60, 70 and 80 years old with a small, medium, large or massive rotator cuff tears.

2.6 Discussion

These results demonstrate that the overall healing rate at 12 months of 220 operations, performed by 65 surgeons in 47 hospitals, was 57%. A review of the literature reveals that 49 studies have reported an overall healing rate of 68% in patients with average age of 60 years. Of these, 21 studies were randomised controlled trials (RCTs), 1 pilot RCT[105], 6 Quasi-randomised controlled trials[106-111]. Of the 28 studies reported as Level 1 evidence (high quality randomised controlled trials), there is still substantial risk of bias. Whilst randomised controlled trials are, in principle, designed to minimise bias from a variety of causes, it is vital that they are conducted and reported correctly to ensure their individual risk of bias is low. By utilising data carefully recorded from a well-designed and conducted randomised controlled trial (UKUFF trial), the risk of bias is minimised. Therefore, the reported healing rate of 57% is likely a closer estimation of the true healing rate. Additionally, the UKUFF trial was more pragmatic in nature than all other level I studies pertaining to rotator cuff surgery. Surgeons were allowed to use their preferred suture anchors, repair constructs, and rehabilitation regime for example. The pragmatic trial design of the UKUFF study provides external validity to a wide range of surgeons treating rotator cuff tears. As such this chapter, as it used data from this trial, also has good external validity for surgeons using these findings in their outpatient clinics, whilst consulting patients with rotator cuff tears.

2.6.1 Reported healing rate by publication date

When considering the healing rate of rotator cuff repair from these 28 studies, the pooled weighted healing rate of rotator cuff repair went down from 94% in 2007 to 71% 2015. There are multiple explanations for this observation. One possibility is publication bias and an increased tendency to report not just clinical outcomes but also imaging of repair integrity post-surgery. In addition, there has been a dramatic increase in the incidence of rotator cuff repair surgery reflecting a probable widening in selection criteria for surgery. Hence, the finding of an overall healing rate of 57% is in keeping with the trend seen with reducing published healing rate over time. The reasons for this trend are yet to be investigated. With an increased use of systematic assessment of patient outcomes following surgery, and raised awareness that not all tears heal despite excellent surgical technique, likely contributed to a widening acceptance to report lower healing rates in the literature.

2.6.2 Age and tear size influence healing rate

Knowing an overall healing rate following surgery is useful. When consulting with patients about the potential outcomes of rotator cuff surgery, a more tailored approach may be useful. Given that patients considering surgery range from 40 to 90 years old, and have anything from a small 1cm tear to a massive 3 tendon tear, it is important to provide a more specific healing rate if these factors influence healing significantly. In this study, patients with a healed repair were 3 years younger on average than those without a healed repair. Additionally, small and medium tears were more likely to heal than large and massive tears.

Assessing whether age and tear size are equally important risk factors, the logistic regression model demonstrated that when age is adjusted for, only massive tears were independently predictive of failure. Park et al (2015), used a univariate analysis in their case-control study to conclude that both age and tear size were prognostic factors for healing. They also noted that the mean age of patients with healed repairs was 59 vs 63 years in those with a failed repair and that tears greater than 2 cm in size healed less often than those less than 2 cm (66% vs. 89%), although the investigators did not adjust for age and tear size together[124].

Lapner et al (2012), found the mean age of those patients with a recurrent tear was 1.8 years older than those with a healed repair, although they concluded this was not significant ($p = 0.479$). Mean coronal and sagittal tear size was statistically significantly different between repairs that healed and failed to heal[32]. Rodeo et al (2012), concluded that at 12 weeks post-repair, 71% of small tears healed compared to 82% of medium tears, and 56% of large tears[147]. Gumina et al (2012), reported that the mean age of patients with a healed repair were 3 years younger than those that had a recurrent tear[141].

In a case-control study, Rhee et al (2014), reported the healing rate in 60-69 year olds as 60%, whilst this reduced to 50% in 70-79 years old patients. They also reported a similar reduction in healing rate with increasing tear size (small/medium tears 85%, large tears 44%, and massive tears 24%)[122]. Two further case-control studies (Kim et al 2012 and Robertson et al 2012) concluded

that the mean age of patients with healed repairs was 61 and 59 years compared to 63 and 61 years in non-healed repairs, respectively[33 123]. Kim et al (2012) concluded this difference was not significant ($p = 0.5$)[33], whilst Robertson et al (2012) made no assessment of the significance of their finding with respect to age[123]. A fourth case-control study concluded the mean age of healed repairs was 59 years compared to 63 years in those that failed to heal ($p = 0.001$)[124].

The predictive model for healing presented in this chapter may be used by surgeons when discussing healing following rotator cuff surgery. Given it estimates the rate of healing at 1 year in patients of different ages and varying tear sizes, it may better guide joint decision-making between patients and surgeons.

2.6.3 Limitations

The potential weaknesses of this work are related to reliance on the UKUFF study data. Whilst this study was well designed and conducted, generating high quality data, the analysis conducted here is beholden to the initial study design that defined the factors on which to collect data. For example, fatty infiltration and muscle atrophy, commonly cited to affect tendon healing, were not systematically recorded in UKUFF. In the UKUFF trial, due to its pragmatic design, surgeons were given freedom to diagnose rotator cuff tears using their preferred pre-operative imaging modality. A variety of different pre-operative imaging systems were used with reporting by different radiologists. Standardised assessment of fatty infiltration and atrophy of supraspinatus muscle was not

possible[203-205 216]. Therefore, it is not possible to investigate the influence of these factors on healing. In the re-analysis of the UKUFF trial data used intra-operative assessment of tear size was carefully standardised, however some variation between surgeons will remain. It is possible that reducing tears to 4 defined size categories improves reliability. However, caution must be exercised when interpreting the predictive model, which requires validation in a separate population. Efforts were made to standardise the postoperative radiological assessment of healing in the UKUFF trial. A single consultant musculoskeletal radiologist, blinded to the type of surgery performed, reviewed all scans. The accuracy of the post-operative imaging assessment was not measured within the UKUFF study.

The strengths of this study of outcomes of rotator cuff repair are its large size, involving 220 patients operated on by 65 surgeons in 47 hospitals. The results represent a real-world evaluation of a commonly performed surgical procedure and are generalisable. Additionally, by using a logistic regression analysis, the individual impact of increasing age and tear size was ascertained.

2.7 Conclusions

In summary, increasing age negatively influenced healing even after controlling for tear size. When controlling for age, only massive tears were an independent risk factor. Clinical decision-making should take the overriding importance of increasing age as a risk factor into account when considering the suitability of rotator cuff surgery for patients. Rates of healing are low even for small tears in

younger patients and there is a compelling case to develop new strategies to improve the success of surgery.

2.8 Biological considerations for rotator cuff healing

Healing following rotator cuff tendon repair is complex. Innovations in surgical implants, sutures, and techniques have demonstrated significant improvements in footprint compression, reduced gapping during cyclical loading, and increased load to failure[66 75 199 211 217]. Unfortunately, the aforementioned findings have not translated to improvements in patient reported outcome measures or healing rate[133 139]. Given incremental advancements in the mechanical properties of rotator cuff repairs have not yielded dramatic improvements in healing, surgeons have attempted to enhance the biological environment for healing[31 132].

2.8.1 Observations in biological changes in rotator cuff tears

Surgeons have previously noted and described the sequelae of chronic rotator cuff tears. Focussing on the rotator cuff muscles, studies have demonstrated differences in the degree of muscle atrophy and fatty infiltration between patients[218 219]. Observational studies have suggested the more severe grades of muscle atrophy and fatty infiltration preclude healing following surgical reconstruction of the tendon-bone interface[103 216]. These muscle changes are thought to be partially reversible if healing following cuff repair occurs, as a result of restoring the biomechanical loading conditions of the native tendon[220].

2.8.2 Rotator cuff repair augmentation

Whilst these observations may be useful, they only describe chronic changes downstream from the site of injury, the rotator cuff tendon. It is the tendon that tears, and in some patients, may lead to muscle changes described previously. Hence, the focus for the rest of this thesis will be investigating strategies for tendon repair augmentation. In light of the conclusions in this chapter, there is a pressing need to innovate strategies to improve the healing rate in rotator cuff repair. Hence, rotator cuff repair augmentation, aimed at improving the biological and mechanical environment, has been proposed to supplement healing at the tendon enthesis. The next chapter will describe the different augmentation strategies currently employed by surgeons, and will focus on investigating the effect of one strategy, biological patch augmentation, on tendon biology.

Footnote

Parts of this chapter have been published:

Acta Orthop. 2017 Dec;88(6):606-611. doi: 10.1080/17453674.2017.1370844. Epub 2017 Sep 7. Increasing age and tear size reduce rotator cuff repair healing rate at 1 year. Rashid MS, Cooper C, Cook J, Cooper D, Dakin SG, Snelling S, Carr AJ.

Chapter 3: Biological patch augmentation in rotator cuff repair

In the previous chapter, a pressing need for improving the healing rate following rotator cuff repair was identified. Despite significant advances in technique and implants, the overall healing rate remains around 60%[24]. Tendon repair augmentation has been proposed to achieve this. This chapter will explore the tissue response of the native rotator cuff tendon after augmentation with two biological patch augments in clinical use in humans undergoing rotator cuff repair surgery.

3.1 Tendon repair augmentation

Tendon repair augmentation strategies fall into 2 categories: cellular therapies and patch augmentation. The commonest employed cellular therapy is platelet-rich plasma (PRP), where patients' whole blood is centrifuged to remove red blood cells. A growth factor and cytokine-rich concentrate is isolated and injected around the repair. This has been extensively studied. Whilst benefit has been demonstrated in observational studies without a control group, and therefore highly liable to bias, several randomised controlled trials have cast doubt on the efficacy of PRP[105 130]. A Cochrane review of randomised controlled trials concluded no clinical benefit of PRP on rotator cuff repair healing[149]. The tissue response of human rotator cuff tendon to PRP may in fact be harmful. One study showed that PRP reduced cellularity and increased apoptosis in subacromial pain syndrome patients[221]. This chapter will focus on an alternative strategy, biological patch augmentation.

3.1.1 Tendon repair with patch augmentation

Patch augmentation involves applying a graft during surgical repair of the torn rotator cuff. Patches may be applied on top of the repaired cuff tendon (onlay), underneath the tendon (interposition), or in a "bridging" fashion to fill the defect between the tendon edge and the bony footprint. These patches may be synthetic or biological. Synthetic patches are usually made from a non-degradable polymer. They are designed to support the repair structurally, and share the load carried by the rotator cuff tendon[160]. Biological patches are marketed as decellularised grafts that become incorporated and vascularised by the native tendon. It is argued that the grafts provide essential matrix proteins for healing[222]. Biological graft sources include those of autologous origin (e.g. fascia lata autograft), cadaveric donors (e.g. dermal allograft), or from porcine tissues (e.g. dermal or small intestine submucosa, xenograft). These biological patches, sometimes called extracellular matrix (ECM) patches, are processed to remove donor cells, and sometimes chemically crosslinked, before sterilisation for clinical use[222 223]. Crosslinking in biological grafts is thought to improve durability by reducing degradation via matrix metalloproteinases as well as improved cell migration, vascularisation, and remodelling[224]. Two popular biological patches, available for clinical use in rotator cuff repair, are GraftJacket (Wright Medical, Memphis, TE, USA) and Permacol (Zimmer Biomet, Warsaw, IN, USA).

3.1.2 Biological patch philosophy

The philosophy behind biological patch augmentation is that the native tendon tissue often lacks capacity to heal to the bony footprint, and that application of a collagen and elastin-rich decellularised graft, that can become integrated with native cells and blood vessels, would improve the healing rate[222 223]. Thus, the aim is to improve healing rate by improving the biological environment.

3.1.3 GraftJacket patch

GraftJacket Regenerative Tissue Matrix (RTM) is a cadaveric human dermis graft that is not crosslinked and undergoes decellularisation by a proprietary process[223]. It was initially developed for use to cover chronic non-healing foot ulcers. It is manufactured by LifeCell Corporation (Branchburg, NJ, USA) and distributed in the UK by Wright Medical (Memphis, TE, USA). GraftJacket is regulated by the U.S. Food and Drug Administration (FDA) as “human tissue for implantation”.

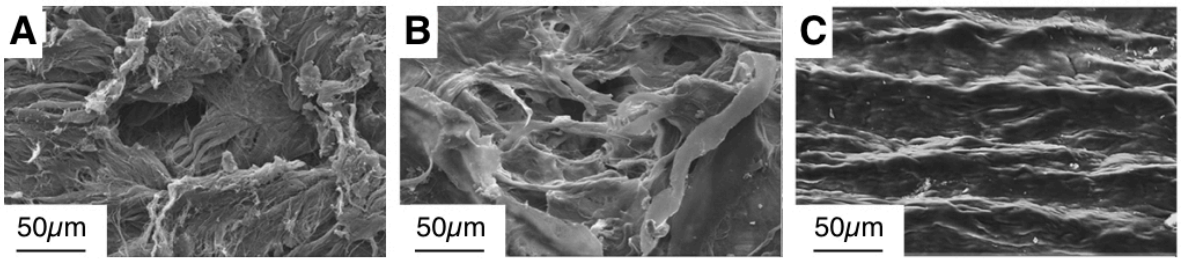


Figure 3-1: Scanning Electron Microscopy (SEM) images of GraftJacket patch, Permacol patch, and normal human supraspinatus tendon. Images show microstructure of these two biological patches compared to that of rotator cuff tendon. Images adapted with Smith *et al* (2017)[225].

3.1.4 Permacol patch

Permacol is a porcine dermis graft that is chemical crosslinked with 4,4′-Diisocyanato-methylenedicyclohexane (HMDI), and is decellularised by a different proprietary process to GraftJacket[222]. It is manufactured by Tissue Science Laboratories PLC (Aldershot, UK) and distributed by Zimmer Biomet (Warsaw, IN, USA) in the UK for orthopaedic applications. It was initially approved for multiple uses in abdominal hernia and genitourinary surgery, sometimes referred to as Pelvicol or ENDURAGen. Permacol was approved for clinical use by the FDA under the 510(k) pathway as a medical device (ref: K021056). Both GraftJacket and Permacol patches are marketed with some supporting information from *in vitro* and animal studies, showing cellular infiltration and neovascularisation however, the mechanisms underpinning these observations remain unclear[224 226-228]. Assessment of the extracellular matrix of both GraftJacket and Permacol patches reveal very different structure when compared to the normal supraspinatus tendon (figure 3-1). The normal supraspinatus tendon has highly aligned fibrils with a characteristic crimp morphology. The processed biological dermal patches have an irregular and disorganised matrix.

3.1.5 Issues with porcine xenograft patches

Porcine small intestine submucosa (SIS) patches are an alternative to dermal grafts. They have decreased in their use following the withdrawal of the Restore Orthobiologic Soft Tissue Implant by Depuy Orthopaedics, INC. (Warsaw, IN,

USA). An abandoned randomised controlled trial comparing rotator cuff augmentation with the Restore patch to a control group (no patch augmentation), yielded two clinical studies demonstrating adverse reactions to the implant[159 186]. Four of the initial 25 patients receiving the patch required open debridement and irrigation[186]. Histopathology of samples taken at the time of debridement showed no microbiological growth, and acutely inflamed granulation tissue. The subsequent cohort study showed a significantly inferior clinical outcome in patients receiving the patch. There was no improvement on healing rate (6/10 in patch group vs 7/12 in the control group) observed at 2 years[159]. This adverse immune reaction is concerning. The *in vivo* tissue response to xenograft and allograft tissue is therefore important to consider in patch augmentation and is the focus of this chapter.

3.2 Aim

1. The aim of this study was to investigate the tissue response of the native supraspinatus tendon to two biological patches at 4 weeks post implantation compared to a control (no patch), using histology and immunohistochemistry.

3.2.1 Hypothesis

1. The null hypothesis was that GraftJacket and Permacol patches do not produce a different tissue response to the control patient group (no patch augmentation), at 4 weeks post-repair.

3.3 Materials and Methods

This study was approved by the NHS Research and Ethics Committee (ref: 15/SC/0697). Patients with confirmed full thickness rotator cuff tears, listed for rotator cuff repair surgery by one of my supervisors (AJC), were invited to participate in the study. Baseline measurements including Oxford Shoulder Score (OSS), Visual Analogue Score (VAS), and EuroQol 5D questionnaire (EQ-5D) were recorded. Patients were divided into 3 groups, Group 1 (GraftJacket augmentation, n=4), Group 2 (Permacol augmentation, n=4), and Group 3 (control – standard repair without augmentation, n=5).

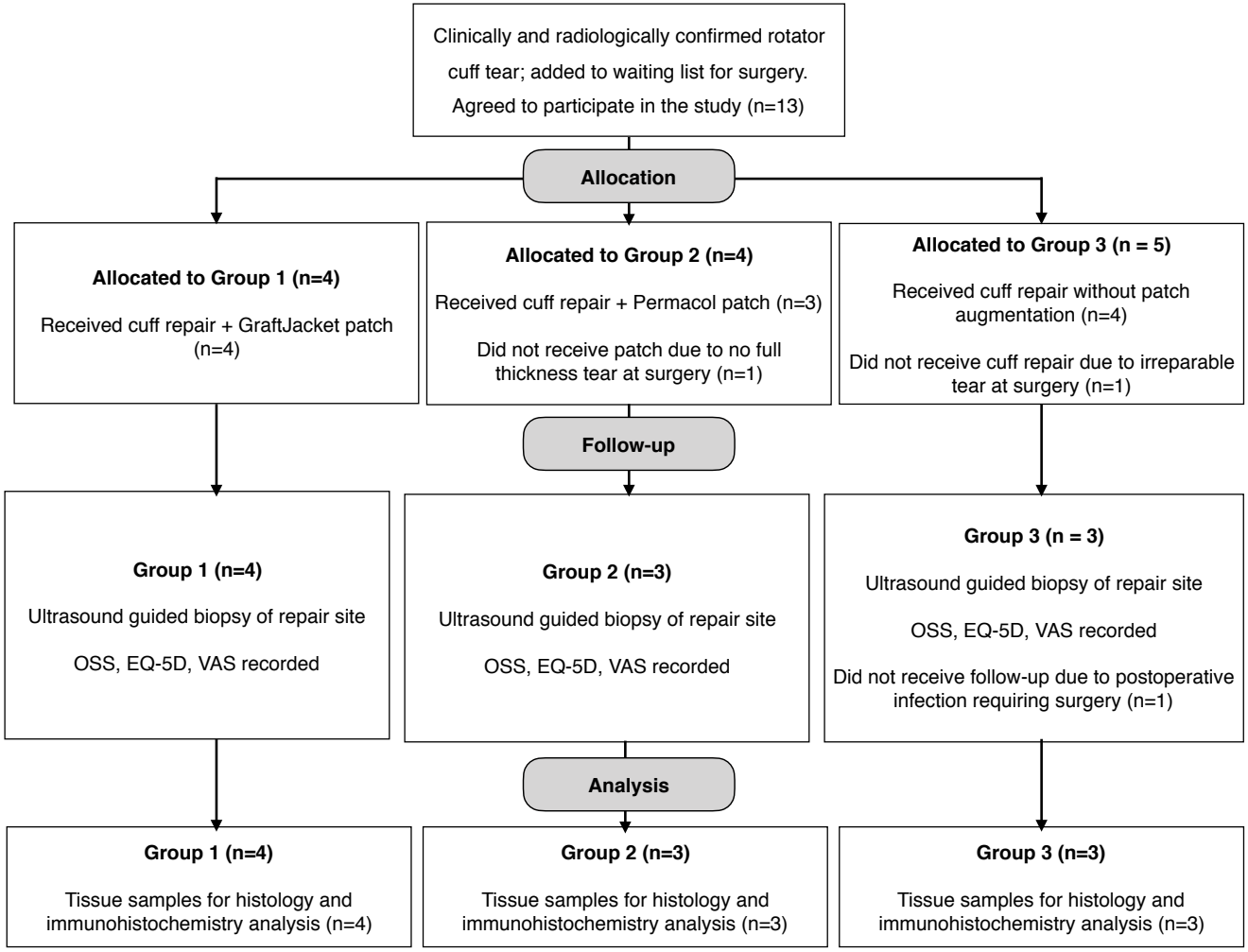


Figure 3-2: Study flowchart illustrating patient group allocation, follow-up at 4 weeks, and tissue analysis. Exclusions and reasons are provided in corresponding boxes.

3.3.1 Rotator cuff repair surgery

Surgical procedures were performed under general anaesthesia with regional blockade (interscalene block). Patients underwent a single row rotator cuff repair with Versalok (Depuy Mitek, Warsaw, IN, USA) and Healix BR (Depuy Synthes, Warsaw, IN, USA) suture anchors via a mini-open, deltoid-splitting, approach. After the repair was completed, patients in groups 1 and 2 had a GraftJacket or Permacol patch respectively, applied in an onlay technique over the repair and secured with 3-0 PDS sutures as per the manufacturers' instructions. Group 3 received a standard rotator cuff repair as above without any patch augmentation.

3.3.2 Rehabilitation and follow-up

Post-operative rehabilitation for all groups was guided by a physiotherapist and included 4 weeks of sling immobilisation, followed by 2 weeks of passive range of motion exercises, and 6 weeks of active mobilisation. At 4 weeks, patients were reviewed in the outpatient setting and repeat measurements of OSS, VAS, and EQ-5D were taken.

3.3.3 Tendon sample harvesting

At the time of surgery, prior to supraspinatus tendon repair, a sample of the torn free edge of the tendon was harvested for histology and immunohistochemistry. At 4 weeks, an ultrasound (USS) guided biopsy under local anaesthesia (10ml 2% lignocaine) of the repaired supraspinatus tendon, was performed with a Bard Magnum core biopsy system and a 16g x 10cm tissue biopsy needle (Bard

Peripheral Vascular, Inc., Tempe, AZ, USA). This technique has been previously validated[229].

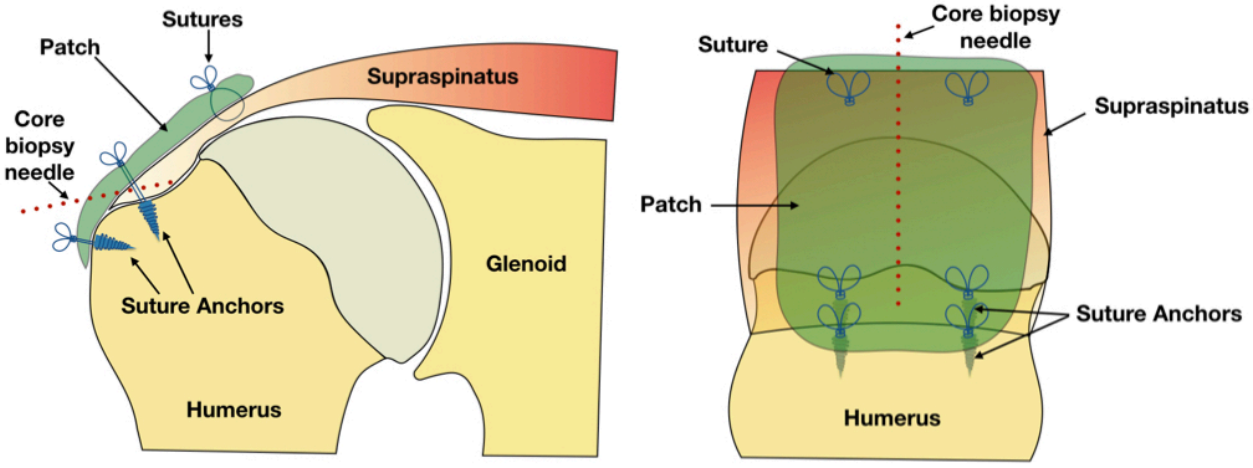


Figure 3-3: Illustrations demonstrating needle biopsy site performed at the 4-week post-operative time point.

3.3.4 Tendon sample handling and processing

All samples were fixed in 10% formalin, processed with a Leica ASP300S tissue processor (Leica Biosystems Nussloch GmbH), and embedded in paraffin wax. Using a rotary RM-2135 microtome (Leica Microsystems Ltd, Milton Keynes, UK), sections of 5-6μm were cut and mounted on Surgipath X-tra Adhesive glass slides (Leica Microsystems Ltd, Milton Keynes, UK). Sections were baked at 60°C for 30 minutes, and then at 37°C for 60 minutes.

3.3.5 Histology and immunohistochemistry

For histology, Haematoxylin & Eosin (H&E) staining was performed using a Tissue Tek DRS automated stainer (Sakura Finetech Europe, Leiden, Netherlands). For immunohistochemistry (IHC), sections underwent high pH, heat mediated, epitope retrieval using a PT Links machine (Dako Agilent Pathology Solutions, Santa Clara, USA). Sections were then stained using a Dako Autostainer Link 48 machine (Dako Agilent Pathology Solutions, Santa Clara, USA).

For immunohistochemistry, antibody staining was performed with the EnVision FLEX visualisation system and an Autostainer Link 48 machine (Dako Agilent Pathology Solutions, Santa Clara, USA), using the primary antibodies listed in Table 3-1. Stained slides were immersed in 90% Industrial Methylated Spirits (IMS) for 3 minutes, 100% IMS for 3 minutes, and then with Xylene solutions for 10 minutes, prior to mounting with DPX mounting medium (Fisher Chemical, Loughborough, UK).

Table 3-1: List of primary antibodies used in immunohistochemistry (IHC), including details and rationale for use.

Primary Antibody	Source	Host	Clonality	Product Code	Conc.	Rationale
CD4	Biorbyt	Rabbit	Polyclonal (IgG)	Orb182470	1:500	Glycoprotein co-receptor on surface of $CD4^+$ T-helper cells.
CD45	LSBio	Mouse	Monoclonal (IgG1)	LS-C187484	1:250	Leucocyte common antigen. Pan-leucocyte marker.
CD68	Dako Agilent	Mouse	Monoclonal (IgG1)	IR609	1:1000	Transmembrane glycoprotein in monocyte lineage cells e.g. monocytic phagocytes.
CD206	Abcam	Rabbit	Polyclonal (IgG)	Ab64693	1:2000	Mannose receptor. Cell surface marker on macrophages and immature dendritic cells.
BMP7	Abcam	Mouse	Monoclonal (IgG1)	Ab54904	1:4000	BMP7 is part of TGFβ superfamily. Counteracts TGFβ1 in fibrosis, anti-fibrotic marker.
IRF5	Protein tech	Rabbit	Polyclonal	10547-1-AP	1:300	Interferon Regulatory Factor 5. A transcription factor expressed by pro-inflammatory macrophages
TGFβ	Abcam	Rabbit	Monoclonal (IgG)	Ab170874	1:150	Cytokine with pro-fibrotic effects

PDPN	Abcam	Mouse	Monoclonal (IgG1)	Ab10288	1:200	Stromal cell activation marker

The choice of primary antibodies for immunohistochemistry reflect a variety of immune cell markers, pro-fibrotic, and pro-inflammatory markers. Each antibody provides insight into the tissue reaction occurring in the native supraspinatus tendon as a result of the overlying patch augment. CD4, CD45, CD68, and CD206 positively stain a range of cells from myeloid and lymphoid lineage[230]. BMP7 and TGFβ are important cytokines with opposing effects on the fibrotic pathway[230]. IRF5 is expressed by pro-inflammatory macrophages, a cell type that may be expected following implantation of a foreign body[230]. Podoplanin (PDPN) is a marker of stromal fibroblast activation. Stromal cell activation has recently been shown to be present in persistently inflamed tendinopathic tissue, although the role of podoplanin in tendon disease is yet to be fully defined[231].

3.3.6 Microscopy, scoring, and image analysis

Stained sections were imaged using a Zeiss AX10 inverted microscope with an Axiom HRc camera and Axiovision software (Zeiss, Cambridge, UK) at 40X and 100X magnification for H&E and IHC respectively. For histology sections, total cell count, foreign body giant cell count (FBGC), and vascularity grading (0-3 scale), were performed on 6 random fields using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Vascularity was graded as follows:

0 = no signs of vascularity;

1 = occasional red blood cells (RBCs) seen, or one small vessel;

2 = several groups of RBCs, or more than one small blood vessel;

3 = extensive blood vessels, one or more large vessel(s), or many RBCs seen in the field of view.

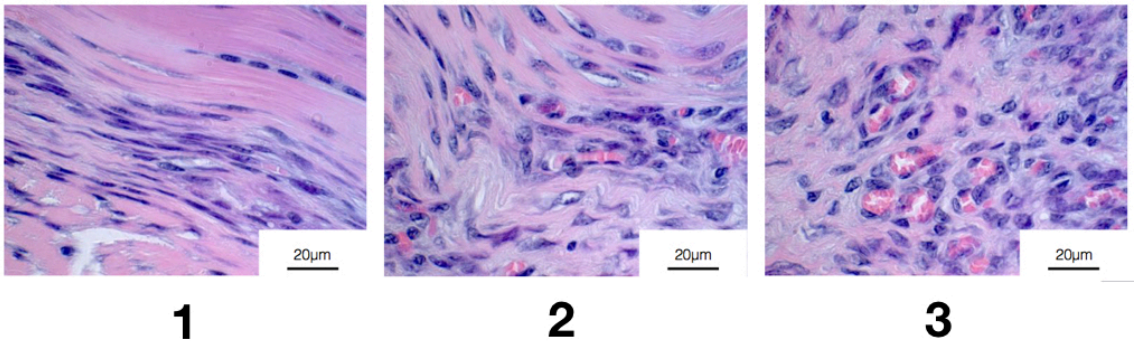


Figure 3-4: Representative H&E stained sections of torn supraspinatus tendon tissue sections. Examples of vascularity grading stages 1-3 are shown. Stage 0 (no signs of vascularity) is not shown.

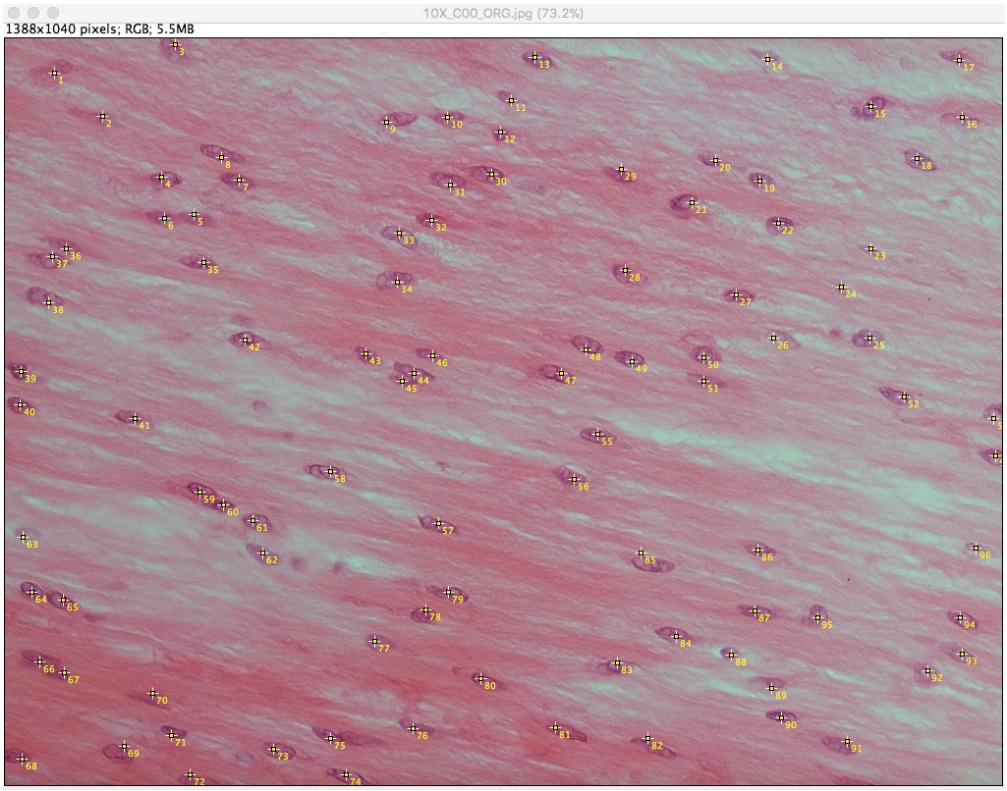


Figure 3-5: Example of cellularity count from a H&E stained section using ImageJ software.

For IHC sections, all images were imported into CellProfiler software (Broad Institute, Cambridge, MA, USA) and a bespoke pipeline was applied to determine percentage of 3,3′-Diaminobenzidine (DAB) staining per nuclei for each sample. For the purposes of analysis, histology and immunohistochemistry results were analysed in groups as described above. Median change from pre-operative to 4 weeks post-operative was used to compare across groups. Statistical analysis using GraphPad PRISM software (GraphPad software, Inc., La Jolla, CA, USA) was performed, where appropriate.

3.3.7 CellProfiler pipeline for quantitative analysis of IHC

After loading images, the 'UnmixColors' module was used to create an image for Hematoxylin analysis and an image for DAB staining analysis. Next the 'IdentifyPrimaryObjects' module was applied to the Hematoxylin image to detect nuclei within the size range of 40-120 pixels using an automatic threshold with an automatic size calculation for smoothing filter. Holes were filled in detected nuclei after declumping by shape. Next, the 'IdentifyPrimaryObjects' module was applied to the DAB stain image to measure DAB staining between 5 and 1000 pixels. A manual thresholding strategy was used with machine learning of 100 random images until a threshold of 0.6 was determined. Size of smoothing filter was automatically calculated and applied. 'Intensity' was selected as the method to distinguish clumped objects. Next, the 'MeasureImageIntensity' module was applied to the areas of DAB staining identified in the previous step. Images from the two main analysis steps were saved and data was exported to a Microsoft Excel file using the 'SaveImages' and 'ExportToSpreadsheet' modules respectively. To calculate the %DAB, the number of pixels of DAB staining from Figure 3-5C (output from 'MeasureImageIntensity' module) is divided by the total number of pixels (1,443,520 pixels) x100. To calculate the % DAB / nuclei, this number is then divided the total object count from Figure 3-5B (output from 'IdentifyPrimaryObjects' module).

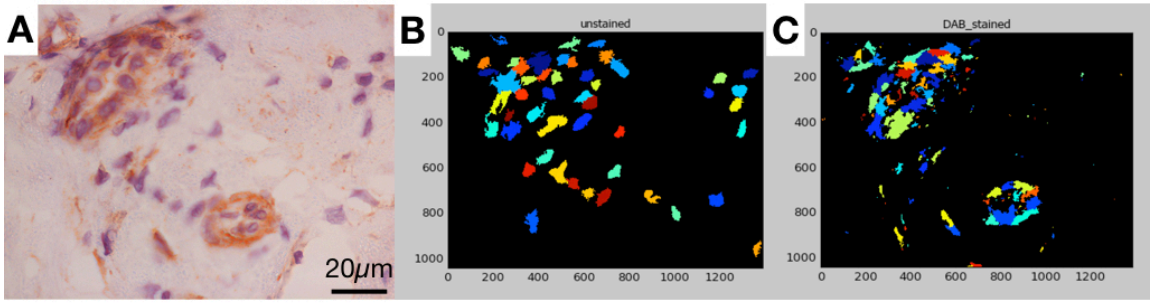


Figure 3-6: Representative images from CellProfiler pipeline. Figures A-C show the various stages of the pipeline. Figure A is an example immunohistochemistry image of (control) patient post-operative section showing DAB staining for anti-BMP7 antibody (brown) and cell nuclei (blue). Figure B is from the 'IdentifyPrimaryObjects' module showing nuclei count performed in this step. Each coloured shape represents a cell. Correlating these shapes with the stained nuclei in figure A demonstrates accurate identification of all calls in the original image. Figure C shows the areas of DAB staining identified by 'MeasureImageIntensity' module. Coloured shapes in this figure accurately correlate to the DAB staining in the original image in Figure A, illustrating accurate identification of DAB staining from this module of the pipeline.

3.4 Results

Thirteen patients were recruited into this study. Three patients were excluded from the tissue analysis. The reasons for exclusion were: partial thickness tear (n=1), irreparable massive tear (n=1), and post-operative deep infection requiring arthroscopic washout and debridement (n=1). The patient with the partial thickness tear had been allocated to the Permacol group and the latter 2

patients to the control group. Thus, 10 patients underwent a rotator cuff repair for a full thickness tear, with tendon tissue samples taken for histological and immunohistochemical analysis pre- and post-surgery. Group 1 (GraftJacket augmentation) included 4 patients; Group 2 (Permacol augmentation) included 3 patients; and Group 3 (control group / no patch augmentation) included 3 patients. Demographics, baseline, and 4 week outcomes of patients are listed in Table 3-2. Due to small numbers in each group, statistical analysis was not performed.

Table 3-2: Study participants. List of patients enrolled in the study including demographic data, patient reported outcome scores (Oxford Shoulder Score, OSS, Euroqol-5D, EQ-5D, and Visual Analogue Scale, VAS) at baseline and at 4 weeks post-surgery. PTT = partial thickness tear. Patients in bold italics were excluded for reasons stated.

Age / Gender	Side	Co-morbidities	Smoker?	Tear Size (cm)	Patch	Baseline OSS (0-48)	Baseline VAS (0-10)	Baseline EQ-5D (0-100)	OSS at 4/52 (0-48)	VAS at 4/52 (0-48)	EQ-5D at 4/52 (0-100)
52	L	Nil	Yes	5	GraftJacket	27	6.9	65	32	2.1	90
49	L	Nil	No	6	GraftJacket	27	5.2	80	15	3.6	85
63	L	Nil	Yes	5	GraftJacket	30	5.7	95	28	1.2	95
55	R	Multiple Sclerosis	Ex-smoker	4	GraftJacket	12	5	85	14	1.6	95
67	R	Hypertension, Asthma	Ex-smoker	4	Permacol	7	10	45	10	-	75
60	R	Nil	No	6	Permacol	28	4.7	96	31	6.1	80
34	R	Nil	Ex-smoker	3	Permacol	41	1.3	70	16	2	45

72	R	Crohn's dx on prednisolone / azithioprine	No	5	Control	15	8.1	75	18	0.1	90
68	L	Hypertension, hyperthyroidism	N/A	3	Control	27	7	80	18	7.6	60
63	R	Hypothyroidism	No	5	Control	18	7.3	90	21	1.3	90
55	***L***	***Asthma***	***No***	***4***	***Control***	***31***	***4.5***	***75***	***13***	***6.7***	***85***
55	***R***	***Nil***	***Yes***	***8***	***Irreparable tear***	***39***	***2.5***	***90***	***41***	***3.2***	***86***
62	***L***	***Hypertension***	***Ex-smoker***	***PTT***	***N/A***	***32***	***3***	***15***	***33***	***-***	***-***

3.4.1 Histology results

Histology using H&E staining demonstrated significant disruption of the extracellular matrix (ECM) in the patch augmentation groups compared to the control group (Figure 3-6 A-C). Specifically, sections demonstrated reduced crimp pattern, increased friability of the matrix, and lack of parallel oriented collagen fibres. Sections from the control group (standard repair without patch augmentation) resembled a similar appearance to normal tendon. Permacol sections had more disruption of the ECM than GraftJacket sections on qualitative assessment. Results for total cell count, foreign body giant cell count, and vascularity grading are presented in Figure 3-7 A-C. There was no significant difference between the groups however, the tissue sections of one patient in Group 2 (Permacol patch) had a distinctly different histological appearance (Figure 3-6 D). This patient was a 67-year-old male with a right sided large rotator cuff tear. Tissue biopsy sections taken post-operatively showed markedly increased cellularity. Morphological features of these cells (rounded cells with large single nuclei and thin cytoplasm with few organelles) suggest they are not tendon fibroblasts, as was seen in other patients' tissue sections, but rather a dense infiltration of immune cells. Clinically, this patient complained of a painful, stiff, shoulder 1 week post-surgery, that settled with analgesia from their general practitioner. Their serum C-reactive protein level at 4 weeks was 10.3mg/L (normal is below 10mg/L).

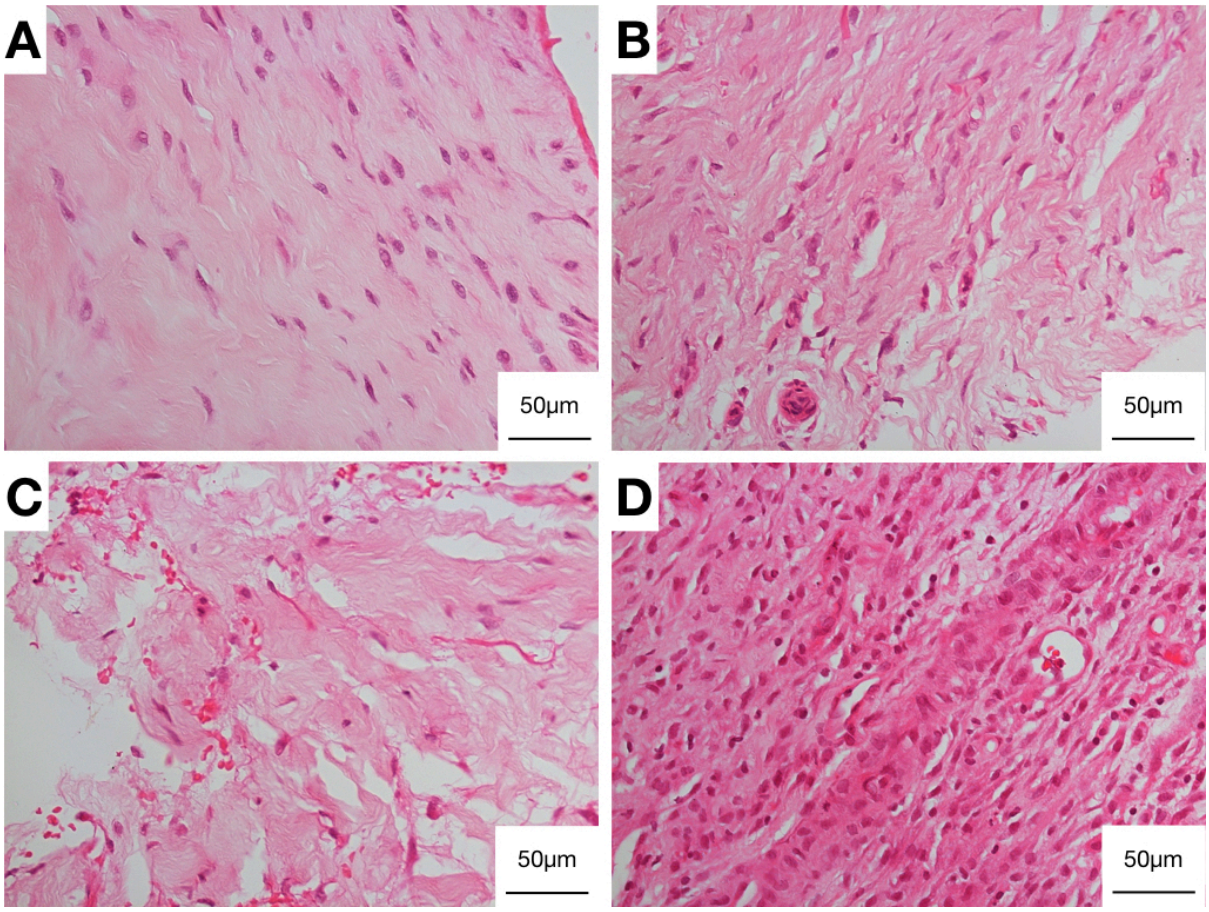


Figure 3-7: Representative histology showing tissue response to patch augmentation compared to control (no patch) group. A-D: Typical 4-week biopsy sections stained with Haematoxylin & Eosin (H&E) for control (A), GraftJacket (B), and Permacol (C) patch augmentation, showing increasing disruption of the tendon extracellular matrix (ECM). D: Abnormal tissue response from patient receiving Permacol showing dense infiltration of immune cells.

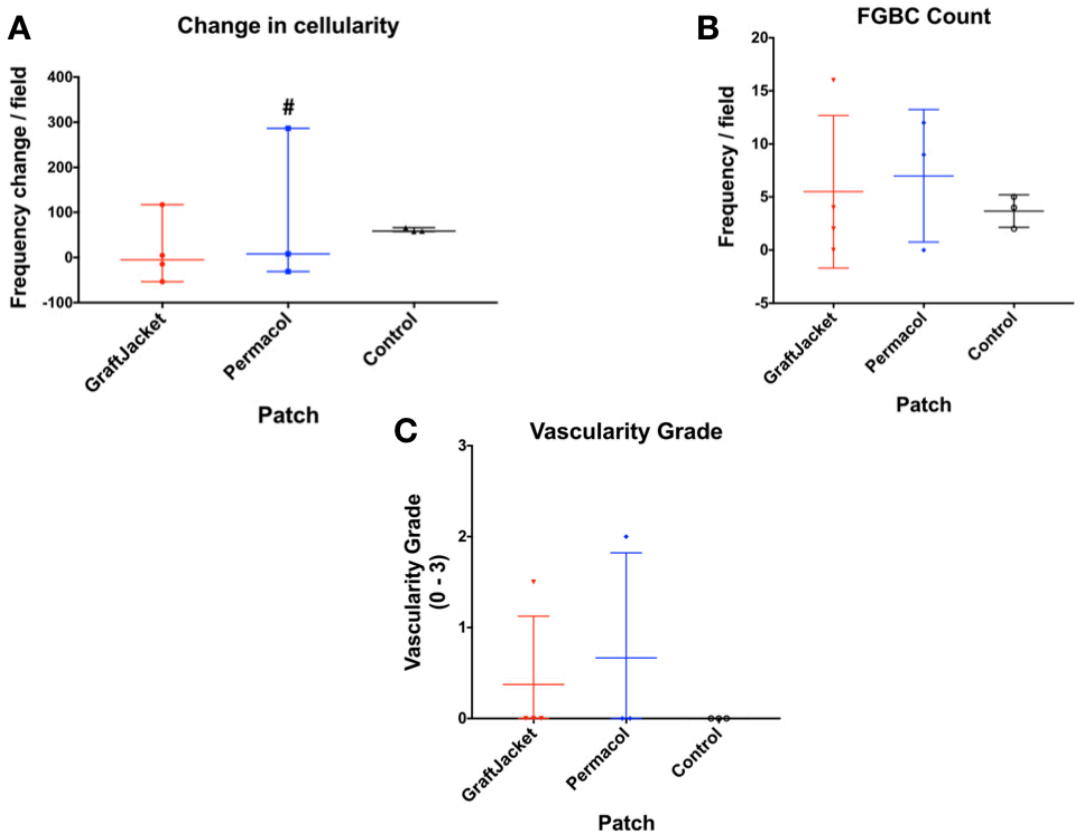
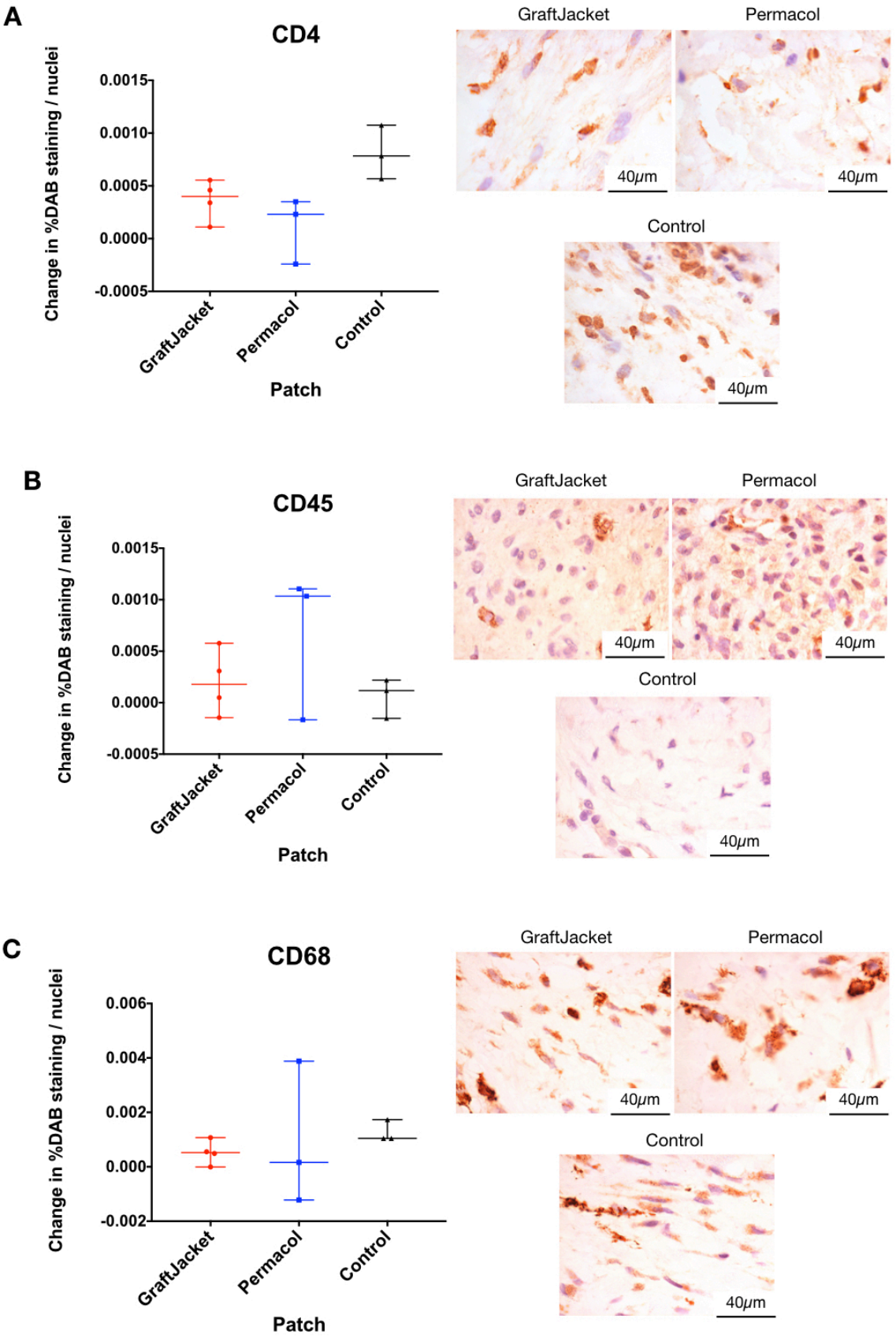
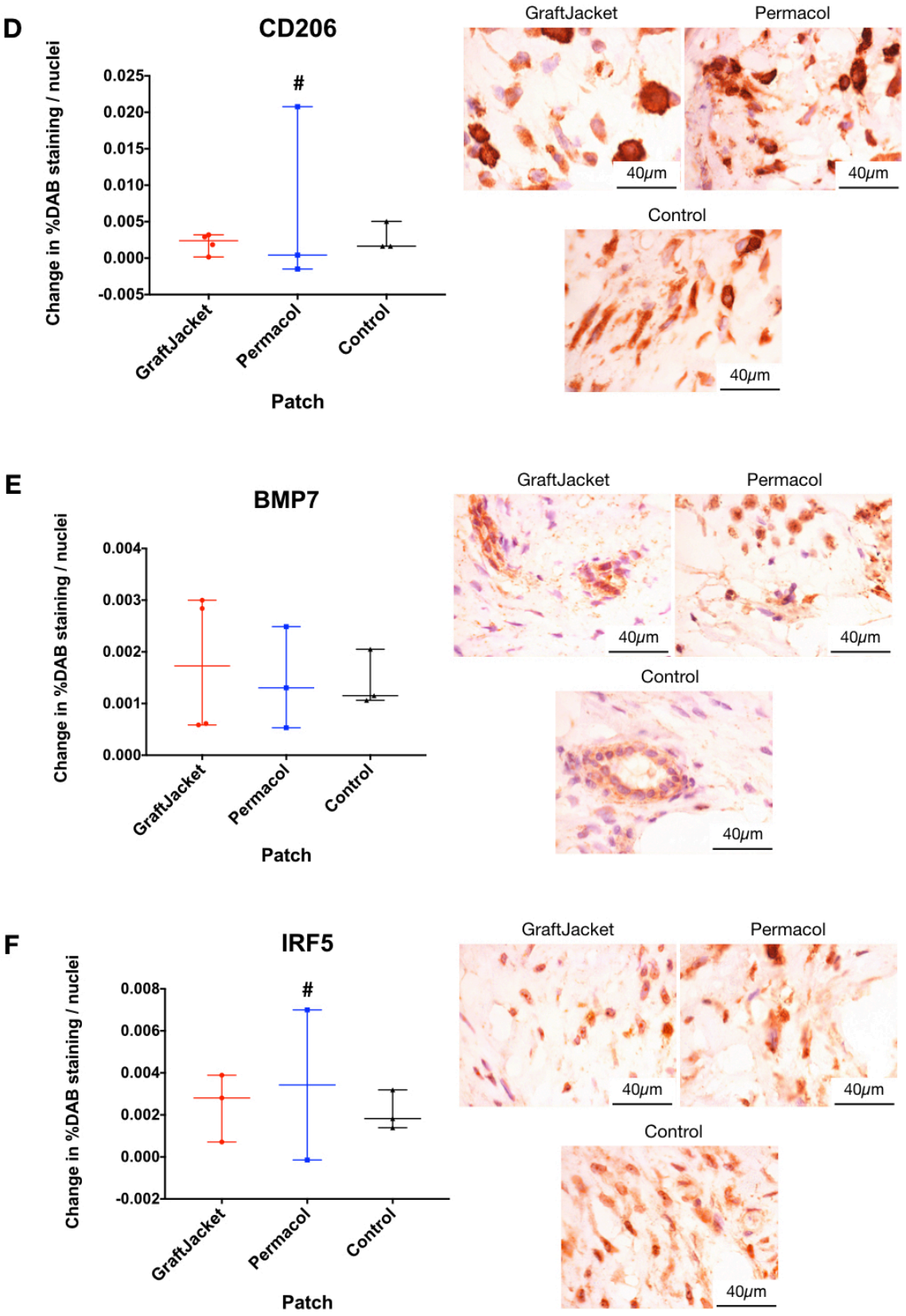


Figure 3-8: Quantitative analysis of histology scoring comparing patch augmentation groups to control. A-C: Histology results comparing three groups (GraftJacket, Permacol, and Control) for change in cellularity (A), Foreign Body Giant Cell (FBGC) count (B), and Vascularity Grade (C). # symbol denotes the patient receiving Permacol with grossly different tissue response.

3.4.2 Immunohistochemistry results

Immunohistochemistry staining revealed no differences between the three groups for the primary antibodies tested (figure 3-8 A-H). However, the patient with the abnormal reaction in Group 2 (Permacol), demonstrated significantly increased immunopositive staining for $CD68^{+}$, $CD206^{+}$, and $IRF5^{+}$ cells (Marked by # in Figures 3-8 C/D/F).


A
CD4
Change in %DAB staining / nuclei
0.0015
0.0010
0.0005
0.0000
-0.0005
GraftJacket
Permacol
Control
Patch
GraftJacket
Permacol
40μm
40μm
Control
40μm
B
CD45
Change in %DAB staining / nuclei
0.0015
0.0010
0.0005
0.0000
-0.0005
GraftJacket
Permacol
Control
Patch
GraftJacket
Permacol
40μm
40μm
Control
40μm
C
CD68
Change in %DAB staining / nuclei
0.006
0.004
0.002
0.000
-0.002
GraftJacket
Permacol
Control
Patch
GraftJacket
Permacol
40μm
40μm
Control
40μm


D
CD206
Change in %DAB staining / nuclei
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
#
GraftJacket
Permacol
Control
Patch
GraftJacket
Permacol
40μm
40μm
Control
40μm
E
BMP7
Change in %DAB staining / nuclei
0.004
0.003
0.002
0.001
0.000
GraftJacket
Permacol
Control
Patch
GraftJacket
Permacol
40μm
40μm
Control
40μm
F
IRF5
Change in %DAB staining / nuclei
0.008
0.006
0.004
0.002
0.000
-0.002
#
GraftJacket
Permacol
Control
Patch
GraftJacket
Permacol
40μm
40μm
Control
40μm

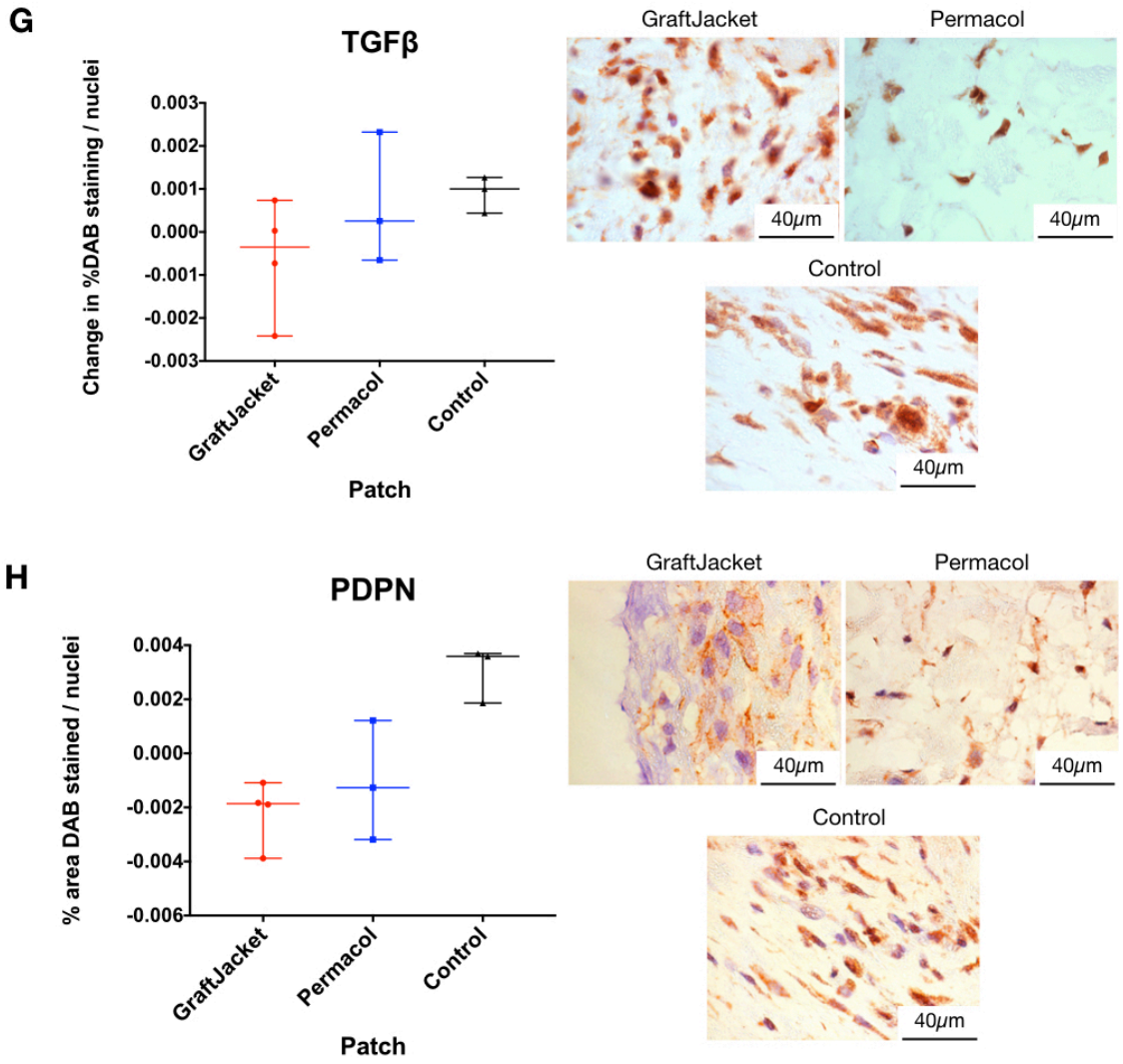


Figure 3-8: Immunohistochemistry (IHC) results showing change in immunopositive (DAB, brown) staining per nuclei (violet) in three groups (GraftJacket, Permacol, and Control). A-H: Comparing change immunopositive staining per nuclei in three experimental groups for various primary antibodies (labelled). # symbol denotes patient receiving a Permacol patch, with a grossly different tissue response, showing significantly higher immunopositive staining against CD68, CD206, and IRF5. Representative images of native supraspinatus tendon sections taken at high 100X magnification are shown.

3.5 Discussion

Patch augmentation is increasingly used in an attempt to improve the healing rate of rotator cuff repair surgery. However, there are currently no clinical studies that utilise post-implantation biopsies to investigate the early tissue response to these grafts. This study is the first to characterise the early *in vivo* tissue response in humans undergoing rotator cuff repair with and without xenograft or allograft biological patch augmentation. Significant extracellular matrix disruption was observed in the native supraspinatus tendon in response to both GraftJacket and Permacol patches compared to the control (no patch augmentation) group. The Permacol xenograft group demonstrated increased ECM disruption of the native underlying supraspinatus tendon, than the GraftJacket allograft group. At the early (4 week) time point, there was no increase in foreign body giant cells or vascularity in both patch groups compared to the control (no patch) group. Whilst there was no difference in cellularity between the groups, one patient in group 2 (Permacol patch augmentation) had significantly increased cellularity assessed histologically from a biopsy sample taken 4 weeks post-operatively. This patient, experienced a painful, stiff, shoulder post-operatively. The tendon tissue sections from this patient were densely infiltrated with immune cells on histological evaluation. Immunohistochemistry did not reveal any significant differences between the groups however, the sections from the aforementioned patient in group 2 showed a significant increase in $CD68^+$ / $CD206^+$ / $IRF5^+$ cells, suggestive of an adverse immune response.

3.5.1 Biomechanical properties of GraftJacket and Permacol patches

Several studies have evaluated the biomechanical properties of GraftJacket and Permacol[232-234]. GraftJacket augmentation vs conventional repair increased load to failure by 55N (309 vs 254N) in one cadaveric study[232] and 215N (560N vs 345N, $p < 0.01$) in another[233]. One study demonstrated Permacol to have a significantly higher shear storage modulus, but similar failure strain and maximum stress than GraftJacket[234]. It is unsurprising that an additional layer of tissue that shares the load with the native repaired tendon will increase the load to failure of the construct, however it is not clear whether the recorded increase is clinically significant, or has any influence on repair failure. Additionally, these grafts require integration with the native tendon over time if this additional biomechanical support is to be sustained, as fatigue failure may occur with cyclical loading over time. This process is a biological one, hence understanding the tissue response over time is also important.

3.5.2 In vitro investigations of GraftJacket and Permacol

The response of tendon cells on GraftJacket and Permacol patches has been investigated in the laboratory[235-237]. Human tendon-derived cells cultured on different patches exhibited different morphology. On synthetic patches, they demonstrated a healthy tenocyte morphology with extended lamellipodia. Cells cultured on GraftJacket were smooth and exhibited filopodia in different directions, whilst cells on Permacol were dendritic and punctate. Compared to

cells growing on synthetic patches, the ratio of Collagen I : Collagen III was lower in biological patches[235]. Rodent rotator cuff tendon-derived cells cultured on GraftJacket showed an increase in prostaglandin levels[236]. Another study quantified the residual donor DNA content in GraftJacket patches, which may result in an immune response after implantation[237]. In addition, all biological patches tested showed significant variance in protein content and residual DNA content[237].

Given commercial patches used for augmentation are sourced from different cadavers or animals, albeit using a standardised protocol for harvesting, processing, and sterilisation, no two patches are ever the same. Hence, the observed immunological response in our study and reported *in vitro* work occurs in the context of this variation on a microscopic level. A sample of patch was not taken for analysis prior to implantation into patients in this study. Therefore, it is not possible to comment on whether the observed adverse immune response in the patient in the Permacol group could be identified by *in vitro* analysis. We do not know what the important variables are that determine host response to an ECM based graft and it is possible that *in vitro* testing would not reproduce or elucidate the clinical findings. A benefit of synthetic patches is that they can be designed for a specific application (e.g. rotator cuff repair). They are not subject to natural variability of harvested tissue as in biological patches, and manufactured to meet strict standards for quality management[237].

3.5.3 In vivo observations using GraftJacket and Permacol patches

In a case report of a 2nd look arthroscopy, for a recurrent rotator cuff tear following primary repair with GraftJacket augmentation, it was noted that two thirds of the graft had incorporated but the anterior one third had detached from the humeral head. The authors also took a sample at the time of surgery, which was used for histology. This revealed widespread infiltration of blood vessels and non-inflammatory stromal cells[238]. Another case series of 4 patients undergoing bridging repair of large rotator cuff tears with Permacol showed very poor results[239]. All four patients failed to improve. Fluid in the subdeltoid bursa was seen in all patients on MRI (one aspirated to confirm sterile effusion). 2 of 4 patients went on to have a reverse total shoulder replacement. At the time of surgery, it was noted that the graft was indistinguishable from the tendon and histology of the debris samples showed chronic inflammation and necrotic fibrous material[239].

In an infraspinatus repair canine model augmented with a human dermal graft, the authors observed infiltration of the grafts by 6 weeks on histology and chronic inflammation. 2 of 10 repairs with the graft had discontinuity at the tendon-bone interface, whilst no defects were seen in the control (no graft) side. The 2 failed repairs demonstrated increased inflammatory infiltrate that the authors concluded this may represent rejection of the graft[240]. The patient receiving the Permacol patch that demonstrated an adverse inflammatory tissue response in the native tendon may have some similarities to the dogs in this

study that had failed repairs. The early immune response to these biological patches may play a significant role in whether the repair heals successfully. A degree of inflammation is integral and conducive to normal tissue healing, however persistent chronic inflammation that fails to resolves is pathological.

3.5.4 Clinical studies using GraftJacket and Permacol in rotator cuff repair

Despite over 1 million implantations of GraftJacket (in a wide range of surgical applications)[223], and over 100,000 implantations for Permacol (mainly in genitourinary and hernia repair surgery)[222], there are no high quality, low risk of bias, clinical studies evaluating their clinical efficacy. There are several clinical studies investigating the application of GraftJacket or Permacol in rotator cuff repair augmentation, all with significant methodological shortcomings. Most studies are observational, all of which demonstrated some improvement in patient reported outcome measures[176 180 182 184 241]. Only one study included a comparator group, reported as a level II randomised controlled trial[106]. This quasi-randomised controlled trial of patients undergoing rotator cuff repair for large posterosuperior rotator cuff tears included 42 patients, randomised to either conventional arthroscopic repair (n=20), or arthroscopic repair plus augmentation of GraftJacket as an onlay (n=22). They observed no adverse events related to the patches, although one patient developed bursitis post-operatively. This study suffers from several limitations and there are serious concerns for risk of bias in trial design, conduct, and reporting, as assessed by the Cochrane risk of bias tool domains[129].

3.6 Limitations

This chapter has limitations that must be considered in light of the results. Firstly, the numbers are small and not appropriate for statistical analysis. Any difference observed in the immunohistochemistry, particularly CD45 and PDPN, may not be robust. There are challenges with recruiting patients to undergo patch augmentation followed by a biopsy of the repair soon after surgery, 4 weeks later and this study was designed to explore the safety and feasibility and performing a tissue based assessment of augmentation grafts in rotator cuff repair. Secondly, with any research involving core biopsies, despite best efforts to standardise, there is some variance with the location of each biopsy. Although all biopsies, guided by ultrasound are of the repair site however, not all samples included both the native tendon and the overlying patch tissue. Hence, it was only possible to investigate the native tendon tissue response. Given that the time point for these biopsies is early (4 weeks), significant cellular infiltration or neovascularisation within the patch tissue at this time is not expected. Third, given the nature of the study, the tissue response was assessed at only one time point. The focus was on the early response as the early phases of healing are considered influential to the overall outcome, and any acute inflammatory reaction would be observed at this time. This study assessed outcomes at one time point, hence temporal changes between the groups cannot be identified. Fourth, there are currently no specific and sensitive cell markers for identifying tendon fibroblasts exclusively. Most commonly used cell markers also stain other immune cell types. Hence, total cellularity in the histology sections was used to

identify differences between the groups. Finally, this study was not designed to demonstrate clinical efficacy of one patch over another. For this, a well-designed, well-conducted randomised controlled trial with specific inclusion and exclusion criteria, and a validated primary outcome would answer the question of whether patch augmentation is superior to a control group (e.g. no patch augmentation).

Both patches are licensed and used widely for rotator cuff repair augmentation, despite initially being developed for other, non-musculoskeletal applications. The human rotator cuff tendon enthesis is complex, and if patch augments are to make significant contributions towards improved healing, a more tailored approach of scaffolds specifically designed for this purpose may be beneficial.

3.7 Conclusions

This is the first study to systematically examine the native tissue response to two commercially available biological patches applied in rotator cuff augmentation in humans. Significant disruption of the supraspinatus tendon ECM was observed in the GraftJacket and Permacol patch augmented groups, compared to the control (no patch) group. The augmentation groups did not show increased cellularity or vascularity of the native tendon. No differences in a variety of immunohistochemical markers for inflammation, fibrosis, and stromal cell activation were found between the GraftJacket, Permacol, and control (no patch) groups. One patient in the Permacol group had an adverse tissue reaction

characterised by extensive infiltration of pro-inflammatory $CD68^+$ / $CD206^+$ / $IRF5^+$ cells. These findings do not support the use of these patches to augment repair of torn supraspinatus tendons.

3.8 Biological versus synthetic patches for augmentation

The concerns of adverse immune reaction and natural variation in the product in biological patches is circumvented by synthetic patches. However, to date, all synthetic patches have been made from polymers, usually non-degradable in nature, and therefore confer no biological advantage. Synthetic patches have been used to share the load of the repaired tendon. The premise being that the native tendon is weak and tears due to a reduced capacity to carry the physiological load. Hence, a patch that shares this load, may be sufficient in the short term to assist the native tendon to heal. Most synthetic patches are non-degradable and hence often become encapsulated like any implanted foreign body. Biological and synthetic patches subscribe to different philosophies for tendon augmentation. Biological patches provide matrix proteins as biological cues for healing, whilst synthetic patches providing predominantly structural support to the native tendon. Each have their advantages therefore, a degradable synthetic patch that can provide mechanical support whilst stimulating a favourable response in the healing tendon is desirable.

3.8.1 Novel synthetic patches providing biophysical cues

The next chapter will describe how a synthetic patch from a biodegradable polymer can be manufactured to biomimic the extracellular matrix microarchitecture of tendons. This patch, produced using a technique known as electrospinning, provides tendon cells with biophysical cues that are advantageous for healing. This patch has been developed and investigated in the laboratory *in vitro*. To determine the *in vivo* response, an investigation of how a surgical defect in a tendon of a large animal responds to this electrospun polymer patch will be undertaken.

Chapter 4: Biodegradable Electrospun Patch for Tendon Augmentation

In the previous chapter, it was demonstrated that biological patch augmentation is not supportive on a cellular level in augmenting tendon repair. An adverse native tendon tissue response to the allograft and xenograft patches tested occurred. Additionally, there are concerns that the inherent variation of the patches, taken from different biological sources, may lead to inconsistent responses. Synthetic patches have traditionally been used to support the biomechanical rather than biological requirements for tendon repair. This chapter focusses on a novel synthetic patch, developed for the purpose of tendon augmentation, designed to provide biophysical cues to tendon cells and enhance endogenous tissue repair. An investigation of the *in vivo* tissue response to this novel synthetic patch is performed and reported in this chapter.

4.1 Current synthetic patches

There are several synthetic patches available for clinical use. Most are non-biodegradable, whilst others degrade very slowly, retaining most of their mechanical strength over a year [242]. Synthetic patches can be designed to have desirable mechanical properties for their specific purpose, such as suture retention[160]. Synthetic patch design has focussed on using “inert” polymers to produce a scaffold that is primarily required to aid the native cuff in carrying physiological load. Table 4-1 outlines some of the synthetic patches used to augment rotator cuff repair.

Table 4-1: Synthetic patches in clinical use for rotator cuff augmentation including manufacturer and polymer substrate.

Name	Manufacturer	Polymer
X-repair	Synthasome	Poly-L-lactic acid
LARS Ligament	Ligament Augmentation and Reconstruction System	Polyethylene teraphalate
Leeds-Kuff Patch	Neoligaments	Polyethylene teraphalate
Biofiber	Tornier	Poly-4-hydroxybutyrate (P4HB)
Artelon	Zimmer Biomet	Poly(urethaneurea)
Biomerix RCR patch	Biomerix	Polycarbonate poly(urethanurea)
Gore-Tex Soft Tissue Patch	WL Gore and Associates	polytetrafluoroethylene
Mersilene mesh	Ethicon	Poly(ethylene terephalate)

4.1.1 Desirable properties of patches for tendon repair

A patch designed to augment rotator cuff tendon repair is one that should possess certain desirable characteristics. Firstly, it must be safe to use in humans. For this particular application, the patch will be positioned in a synovial environment, and attached to tendon and/or bone, depending on how it is

applied. Thus, any synthetic material must be compatible with coming into prolonged contact with these tissue types.

The initiation of rotator cuff tendon tissue tears can be related to a traumatic event or be atraumatic in nature. Atraumatic tears may be related to repetitive physiological loading in a tendon that has been weakened by recurrent episodes of inflammation and fibrosis. Therefore, patches must be stronger than the native torn tendon. One may argue that mechanical properties should be akin to normal healthy tendon, or perhaps even stronger than normal tendon to avoid the potential of implant failure. One concern of patches being significantly stronger than normal tendon, is of a risk of unloading the underlying tendon, by carrying all of the load through the patch. This is borne out of a hypothesis that an unloaded tendon undergoes adverse biological changes[243].

Another desirable characteristic is that of biological activity. Many synthetic patches have been designed with the biomechanical environment in mind. Some have been designed to allow for vascularisation of the patch over time, although these claims are so far unsupported clinically[244]. Any foreign material, regardless of how inert it's substrate is, will produce a reaction in situ. Even a completely "inert" patch that does not produce an adverse acute immune reaction, will become encapsulated over time by fibrous tissue. The foreign body response to synthetic biomaterials was first detailed in the 1980s by James Anderson[245 246]. The immune system is the first responder to a biodegradable implant, initiating

an acute inflammation phase characterised by polymorphonuclear cells, which precedes a chronic inflammation phase in which macrophages phagocytose implant debris, and fuse to form foreign body giant cells. Granulation tissue forms around the implant and matures into a fibrous capsule over time[247].

Synthetic materials may be designed to provide biophysical cues to the tissue there are applied to, in order to promote a desirable biological response. The microarchitecture of the tendon matrix can be reproduced in intelligent scaffold design utilising strategic biomimicry[248] allowing cells to attach, and consequently change their cell behaviour. Patches designed for tendon repair may target desirable properties to promote fibroblast activity, including synthesis of collagen I. Particularly in the rotator cuff tendon, a generally hypovascular tissue, patches that enhance tissue integration and neovascularisation are desirable.

Finally, once a patch has encouraged the native tendon to heal, it may be desirable for this patch to biodegrade, preventing any long term foreign body reaction. Degradation products however, must be non-toxic to the local cell population, and not induce an immune reaction themselves.

4.2 Electrospinning technology

Electrospinning is the method of applying an electrostatic force to a dissolved polymer solution, producing drawn fibres of micro and nanoscale. In brief, a polymer is dissolved into a solution and placed into a syringe with a nozzle. This

polymer is slowly extruded from the nozzle at a constant flow rate, usually by a mechanical pump. Some distance away from the nozzle, an electrode is placed to provide a charge to the nozzle. Between the nozzle and the electrode is a means of collecting the electrospun polymer fibres that are drawn from the nozzle by the electric charge. A rotating drum collector allows for a layer of fibres to be collected onto a surface. By moving the nozzle back and forth in a horizontal direction, and varying the speed of the collecting drum, fibres collected may be aligned or randomly orientated. The length of time for electrospinning without replacing the surface of the collecting drum will increase the thickness of the layer, as more fibres gather onto each other.

Adjustment of the polymer solution concentration, flow rate, electrostatic force, distance between nozzle and collector, and collector properties will alter fibre properties. Hence, specific material properties can be adjusted and tested in the laboratory. Thus, electrospinning is versatile and allows for a number of applications in biomaterials manufacturing. For polymer-based patches for tendon augmentation, electrospinning may allow production of single or multiple layers of fibres in various pre-determined orientations. Unlike extruded macroscale polymers, woven to produce a synthetic patch, such as the Leeds-Kuff patch, a layer of electrospun polymer fibres is generally very weak and may often require structural support to form a useable augmentation patch for tendon repair.

4.2.1 Development of an electrospun PDO/PCL patch

A synthetic biodegradable patch made of aligned electrospun fibres and mechanically reinforced by a woven PCL monofilament mesh has recently been developed by our group[190]. Electrospun micro- and nanoscale fibres have been shown to provide biophysical cues to cells from numerous tissues including tendon, ligament, and bone[249-251]. The biomimetic properties of these fibres can be harnessed by design, to mimic normal extracellular matrix microarchitecture.

4.2.2 In vitro and in vivo studies of electrospun PDO/PCL patch

An *in vitro* study suggests this electrospun PDO/PCL patch promotes mesenchymal stem cell differentiation, tendon-derived cell attachment and cellular proliferation[252]. A previous study evaluated the *in vivo* biocompatibility of the PDO/PCL electrospun patch in a rodent infraspinatus repair model[190]. An increase in foreign body giant cells FBGCs), peaking at 4 weeks, and reducing to negligible quantities by 6 weeks was observed. Compared to vicryl scaffolds, there was a significant reduction in FBGCs at 6 weeks. As the electrospun layer began to degrade, significant cellular infiltration of well orientated tendon fibroblasts was seen. Additionally, staining for M1 and M2 macrophage subtypes (anti-iNOS and mannose receptor respectively) showed no increase in classically and alternatively activated macrophages at any time point compared to a suture only repair (control)[190]. Well orientated cells are seen in line with aligned electrospun fibres, thought to be tendon fibroblasts[190]. No adverse events were noted in this study of 60 Lewis rats.

4.3 Animals models for tendon repair

To further investigate the potential for electrospun PDO/PCL patches to augment tendon repair, these scaffolds were implanted into a previously validated ovine model of tendon injury within a synovial fluid compartment[253]. Many small animal models for tendon healing fail to adequately represent the equivalent human condition, as these studies frequently use skeletally immature animals with spontaneously healing tendon lesions[254]. The ovine tendon injury model utilised in this chapter induces repeatable tendon defects within a synovial environment in an adult animal. These defects do not heal spontaneously, analogous to human tendons that require surgical intervention.

4.4 Aims

The aims of this study were to investigate if, using a validated ovine model of tendon injury, electrospun PDO/PCL patches:

a) induce tendon repair,

b) are safe to use

c)augment tendon healing without producing a significant systemic or local foreign body tissue response.

4.4.1 Hypotheses

1. Electrospun PDO/PCL patches induce tendon healing in an ovine model
2. Electrospun PDO/PCL patches are safe

3. Electrospun PDO/PCL patches do not produce a systemic response, or adverse local foreign body response.

4.5 Materials and methods

4.5.1 Patch manufacturing and assembly

Electrospun PDO/PCL patches have been developed prior to the start of this DPhil and were fabricated by Antonina Lach* using a method previously published[190 255]. The steps of manufacture are summarised in the following steps.

4.5.2 Production of electrospun polymeric layers

Polymer solutions were prepared by dissolving polydioxanone (PDO, 1.5–2.2 dl/g, Evonik, Essen, Germany) or polycaprolactone (PCL, Vornia, Dublin, Ireland) into 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Apollo Scientific Limited, Cheshire, UK) at concentrations of 11% and 20% respectively (weight to volume ratios). Solutions were agitated at room temperature on a roller for at least 24h to allow for complete dissolution of the polymers. Polymer solutions were electrospun with a temperature and humidity-controlled electrospinning machine (IME technologies, Geldrop, Netherlands). PDO electrospun meshes were produced by electrospinning the PDO solution for 2h onto a drum rotating at 2000 rpm with a voltage of 8.2 kV. PCL electrospun meshes in two

*Antonina Lach is a member of the Carr research group and is a biomanufacturing technician with expertise in electrospinning scaffolds. (https://www.ndorms.ox.ac.uk/team/antonina-lach)

configurations: (1) as aligned meshes by electrospinning the PCL solution for 2h onto a drum rotating at 100 rpm and a voltage of 8.4 kV, (2) as thin grids by electrospinning onto a thin nickel mesh (Precision Micro, Birmingham, UK) for 15 mins at a voltage of 8.4 kV. For all experiments, the nozzle-collector distance was set at 20 cm, the temperature was maintained at 21˚C, and humidity maintained at 30%. All samples were stored in a desiccator prior to use.

4.5.3 Weaving

The woven monofilament PDO layer was created with a twill weave. A twill weave is one whereby the weft thread is passed over one or two warp thread then under one or two ward threads, creating a characteristic diagonal pattern[256]. Twill weave structures were produced from PDO monofilaments (7/0, Riverpoint Medical, USA) using an industrial loom (model NH2 53, Jakob Müller AG, Frick, Germany).

4.5.4 Patch assembly and cutting

A PCL aligned mesh was sandwiched between the PDO woven component and a non-woven electrospun PDO layer. The components were then maintained together under a gentle pressure on a hot plate at 80˚C for 1-3 min to melt the PCL mesh (Tm = 65˚C), acting as an adhesive following solidification upon cooling. Similar steps were then used to attached an additional 6 layers of PDO aligned materials using the PCL grids. Following layering, samples were cut to

shape (5 mm x 10 mm) using a hot wire cutter (Proxxon Thermocut 230/E, Wecker, Luxemburg) to melt edges and avoid fraying.

4.5.5 Scanning Electron Microscopy (SEM)

Both sides of the patch (top electrospun layer and woven layer) were analysed after gold coating using a SC7620 Mini Sputter Coater System (Quorum Technologies Ltd, East Sussex, UK) by scanning electron microscopy (SEM) using a Zeiss Evo LS15 Variable Pressure Scanning Electron Microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Images were taken at 5000X magnification to allow a minimum of 30 fibres from 3 images to be measured for their diameter. The median fibre diameter was calculated in nanometers (nm).

4.5.6 Surgical procedures and macroscopic observations

The study was performed under UK Home Office license (PPL 70/6105) and with approval from the Ethics and Welfare Committee of the Royal Veterinary College (RVC). A previously validated ovine model of surgically induced tendon injury was utilised to investigate the effect of implanted electrospun scaffolds to augment tendon repair [253]. For this study, 8 female, skeletally mature, English mule sheep underwent surgery by Prof. Roger Smith (Veterinary Surgeon). All surgeries were performed under general anaesthesia with intravenous injections of 2% xylazine (Rompun; Bayer Healthcare, Newbury, UK), ketamine (Ketaset; Fort Dodge Animal Health, Kansas, USA) and midazolam (Hypnovel; Roche, Burgess Hill, UK). Anaesthesia was maintained with ~2% isoflurane gas (IsoFlo,

Abbott Labs, Maidenhead, UK) to effect. After aseptic preparation, the lateral digital flexor tendon sheath immediately distal to the metacarpophalangeal joint was opened. A hook knife was used to create a 10mm long, 2mm deep longitudinal cut in the lateral branch of the deep digital flexor tendon. The palmar annular ligament and the surrounding superficial digital flexor tendon was transected longitudinally to allow the deep digital flexor tendon at the site of the defect to be elevated using a hook. The patch scaffold was then applied over the tendon defect, and secured using four 4-0 PDO simple sutures in each corner of the patch. The skin incisions were closed and then the limb was bandaged before allowing the sheep to recover from general anaesthesia. The sheep were initially housed in individual pens but then grouped in large pens which allowed free exercise from 1 week after surgery. They were visually assessed daily for lameness.

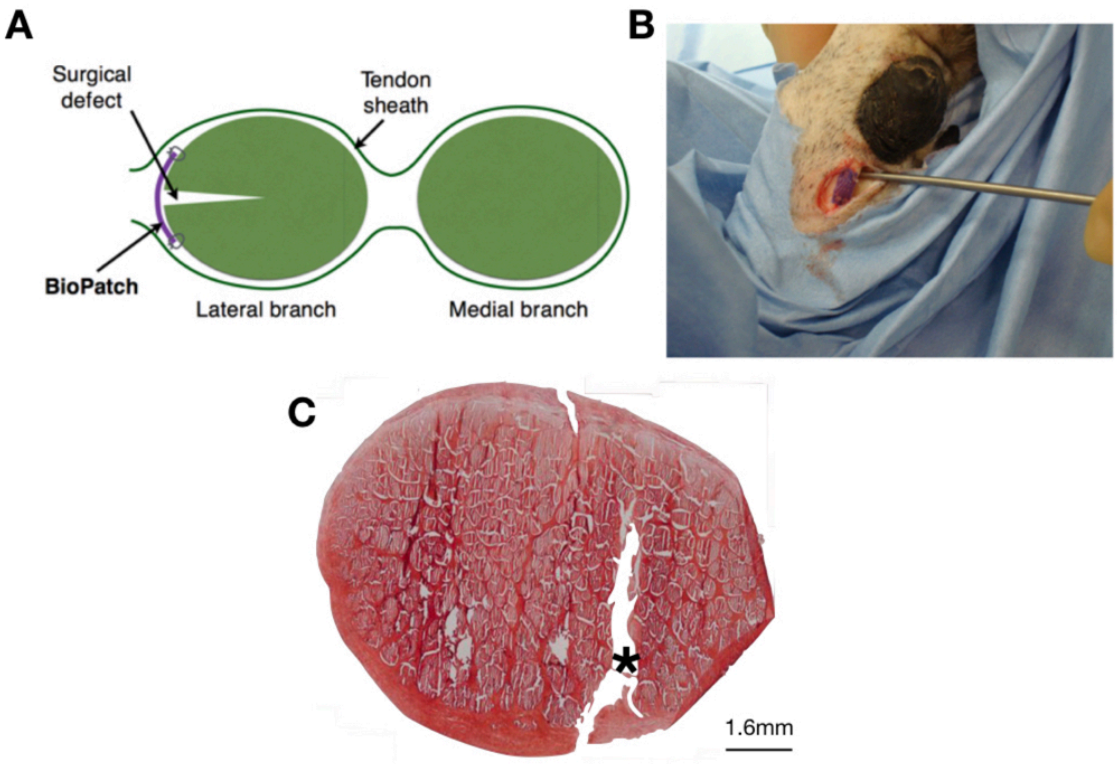


Figure 4-1 Electrospun PDO/PCL patch augmentation in ovine tendon injury model. A: Diagram showing cross-sectional view of the ovine deep digital flexor tendon (and enclosing digital flexor tendon sheath), demonstrating the surgical defect and the repair with patch (violet). B: Photograph demonstrating patch applied over surgical defect on lateral branch of right forelimb deep digital flexor tendon. C: Low magnification (2.5X) bright field microscopy image of H&E staining showing cross section of non-healed DDFT tendon injury, 3 months after surgery [253].

4.5.7 Euthanasia and tendon sample harvest

At 3 months, all sheep were euthanised by overdose of pentobarbitone at a dose of 1ml/kg body mass. Necropsy was performed in all sheep according to

standard operating protocols. During tendon harvest, all repairs were examined macroscopically for signs of tissue healing, residual inflammation, synovial fluid changes, and adhesion formation. The general appearance of the surrounding deep digital flexor tendon (DDFT) sheath and adjacent superficial digital flexor tendon (SDFT) was also recorded. Circumferential measurements proximal and distal to the metacarpophalangeal joint (MCPJ) were made to qualitatively assess swelling of the tendon repair site.

4.5.8 Haematology and serum inflammatory markers

Blood samples for serum preparation were taken from all sheep pre-surgery and at 3 months post-surgery. Full blood count and serum inflammatory markers (serum amyloid A and fibrinogen) were performed by the clinical pathology laboratory at the Royal Veterinary College (RVC).

4.5.9 Histology

The operated lateral branch of the harvested DDFT was cut at the tendon bifurcation and fixed in 10% formalin for 7 days. Tendon samples were then processed using a Leica ASP300S tissue processor and embedded in paraffin wax. 5-7μm sections were cut using a rotary RM-2135 microtome (Leica Microsystems Ltd, Milton Keynes, UK), and placed onto Surgipath X-tra Adhesive glass slides (Leica Microsystems Ltd, Milton Keynes, UK). Slides were baked at 60^{o}C for 30 minutes then 37^{o}C for 60 minutes. Histology with haematoxylin & eosin (H&E) staining was performed. Using a Zeiss AX10

inverted microscope with an Axiom HRc camera and Axiovision software (Zeiss, Cambridge, UK), bright field microscopy images were taken at high (100X) magnification in areas relating to the electrospun fibre zone (ES), and normal tendon zone (NT).

In 6 random images per field, per sample, histomorphometric features were used to record immune cell count (ICC), fibroblast cell count (FCC), and foreign body giant cell count (FBGCC). Vascularity was graded as follows:

0 = no signs of vascularity;

1 = occasional red blood cells (RBCs) seen, or one small vessel;

2 = several groups of RBCs, or more than one small blood vessel;

3 = extensive blood vessels, one or more very large vessel(s), or many RBCs seen in the field of view.

To evaluate the repeatability of this scoring system, two assessors were asked to record the immune cell count, fibroblast cell count, foreign body giant cell count for 20 representative images. Assessors were blinded and recorded their observations independently. Repeat recordings from both assessors were performed 2 weeks later with the representative images reordered randomly using an online random number generator (random.org). Interclass coefficient ratios were calculated between assessors, between time points, and between categories.

4.5.10 Immunohistochemistry

Immunohistochemistry staining was performed for all patch augmented tendon samples. Heat-induced, high pH epitope retrieval was performed using a PT Link machine (Dako Agilent Pathology Solutions, Santa Clara, USA). Antibody staining was performed using the EnVision FLEX visualisation system with an Autostainer Link 48 (Dako) using anti-CD4 (a glycoprotein co-receptor on the surface of T-helper cells), anti-CD14 (a co-receptor found mainly on monocytes), anti-CD45 (a pan-leucocyte cell marker), and anti-TGFβ (a cytokine with pro-fibrotic effects) antibodies (further details are listed in Table 4-2). Antibody binding was visualised using FLEX 3,3′-diaminobenzidine (DAB) substrate working solution and haematoxylin counterstain (Dako) using the recommended manufacturer protocols. Images were acquired using the same microscope-camera system as above, at high magnification(100X) in several fields corresponding to areas relating to the electrospun fibre zone (ES), and normal tendon zone (NT). Immunopositive staining was determined and quantified as the proportion of DAB stained tissue (% of total area) divided by the number of nuclei present (to account for cellularity). All images taken in the electrospun fibre zone were compared to all images taken in the normal tendon zone.

Table 4-2: Primary antibodies details used in immunohistochemistry staining for patch implanted ovine tendons.

Anti-	Source	Host Species	Clone / ID	Dilution
CD4	Biorbyt	Rabbit	Poly / orb182470	1:1000
CD14	Abcam	Mouse	Tuk4 / ab27545	1:100
CD45	LS Bio	Mouse	1.11.32 / LS-C187484	1:200
TGFβ	LS Bio	Rabbit	Poly / LS-C352937	1:150

4.5.11 Statistical methods

Descriptive statistics included median values and 95% confidence intervals (CI). Comparisons between zones (ES and NT), using median values for immune cell count, fibroblast cell count, foreign body giant cell count, and vascularity were performed. A Mann Whitney U test was applied to determine the differences between fields (ES and NT) in the 4 categories (ICC, FCC, FBGCC, and vascularity) using Prism v7 (GraphPad, La Jolla, CA, USA). For the immunohistochemistry (IHC) analysis, a Mann Whitney U test was applied to detect differences between the median percentage DAB staining per nuclei between the electrospun fibre (ES) zones and normal tendon (NT) zones for all primary antibodies tested in all sheep. The level of statistical significance was set at $p<0.05$.

4.6 Results

4.6.1 Scaffold morphology of electrospun patches

Scanning electron microscopy of the electrospun layer demonstrated aligned fibres in a crimp morphology. The median fibre diameter of the electrospun fibres in the electrospun layer was 980nm (Interquartile range, IQR 802 –1323nm). The woven PDO layer demonstrated a twill weave pattern as expected. Median fibre diameter of the woven PDO monofilament was 118μm (IQR 114 - 134μm) (Figure 4-2).

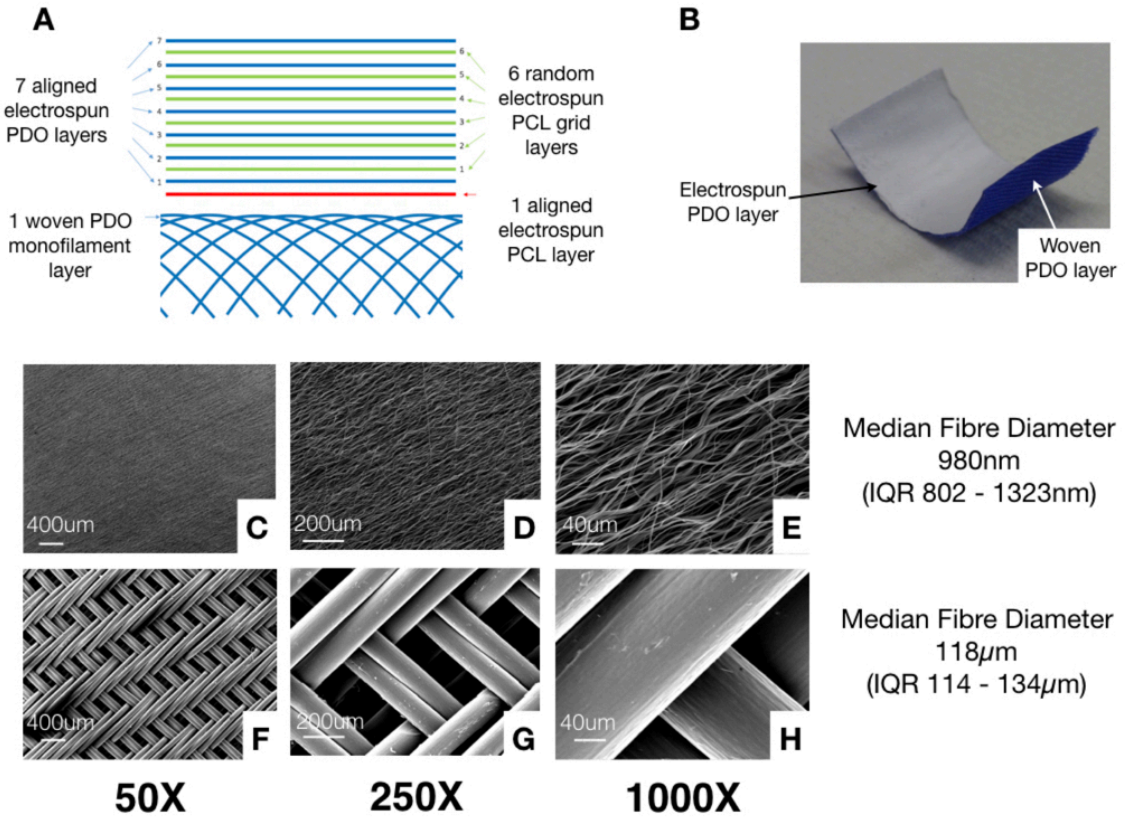


Figure 4-2: Electrospun PDO/PCL patch composition and characterisation. A: Diagram representing different layers in the electrospun patch. Seven layers of aligned electrospun PDO fibres are sandwiched between 6 layers of thin PCL electrospun grids (acting as an adhesive) in an alternate manner forming the electrospun component of the patch. The electrospun component is bound to a woven PDO monofilament layer by a single layer of aligned electrospun PCL fibres. B: Photograph of patch sample showing white electrospun PDO layer (facing tendon) and blue woven PDO layer. C-E: Representative scanning electron microscopy (SEM) images showing electrospun layer. F-H: Woven layer of the patch at various magnifications shown.

4.6.2 Macroscopic observations of electrospun scaffold implanted tendons

There was no difference in the circumference of the forelimb around the metacarpophalangeal joint MCPJ (above and below the ergot) measured pre-surgery and 3 months later, in all sheep (Table 4-3). Macroscopic inspection of the lateral branch of the deep digital flexor tendon after removal of the tendon demonstrated an absence of any defect in all sheep 3 months after patch implantation. There was no residual polymer from the electrospun scaffolds seen on the surface of the tendon in any specimen.

Table 4-3: Results of circumference measurements (in cm) of the right forelimb of the sheep taken above and below the ergot pre-surgery and pre-necropsy / 3 months post-surgery.

	Pre-surgery		3 months post-surgery	
Ref.	MCPJ circumference - Proximal (cm)	MCPJ circumference - Distal (cm)	MCPJ circumference - Proximal (cm)	MCPJ circumference - Distal (cm)
6468	11.0	11.0	13.0	13.0
6469	11.0	10.5	12.0	12.5
6470	11.0	10.5	11.5	12.0
6471	11.0	11.0	12.0	12.5

6472	11.5	11.0	12.0	11.5
6473	12.0	11.5	12.5	13.0
6474	12.0	11.0	13.0	12.0
6475	11.0	10.5	13.0	11.5

A smooth pink-red layer, superficial to the tendon, overlying the healed defect, was present. This layer was indistinct from the underlying tendon and appeared to be fully integrated with host tissue. It appeared softer, and more vascular than the tendon. This layer was rarely adherent to the surrounding sheath. When mild adhesions were observed, the DDFT was easily separated from the surrounding sheath without the need for sharp dissection. There was minimal (<0.5ml) synovial fluid which had a normal appearance (clear, straw-coloured, viscous fluid), and a mild local inflammatory reaction in the surrounding synovium (Figure 4-3).

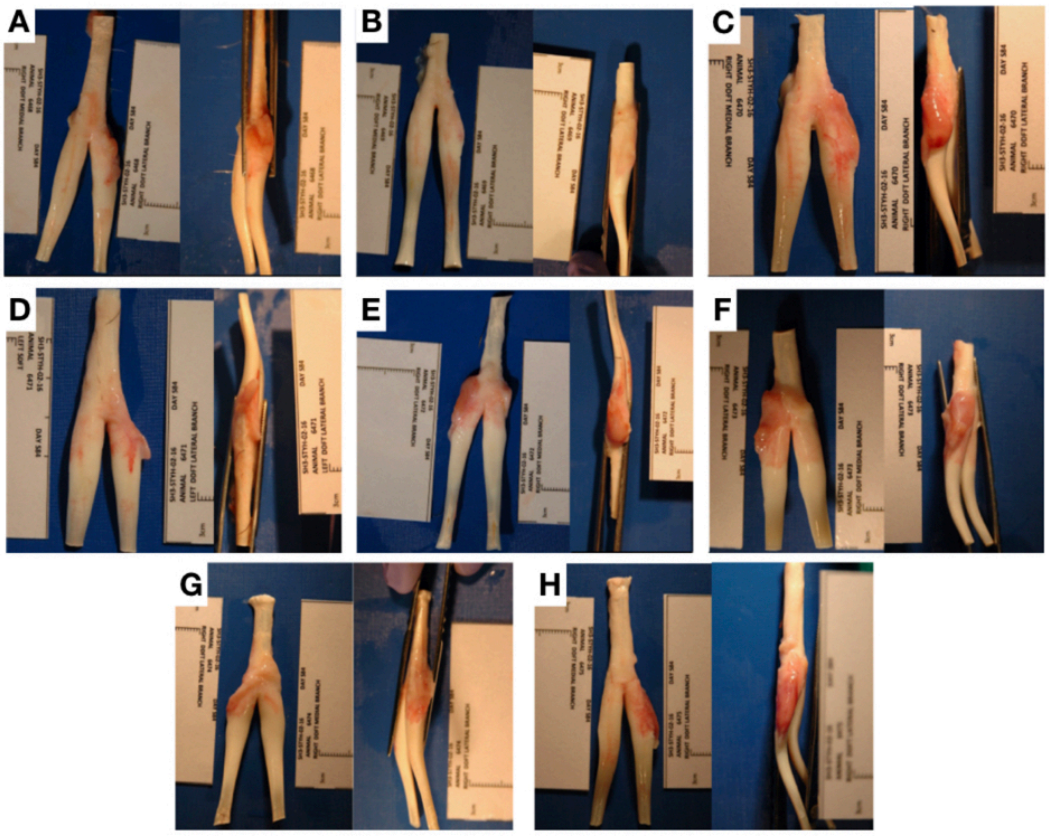


Figure 4-3: Digital photograph images showing harvested tendons augmented with patch scaffolds. Sheep 6468, 6469, 6470, 6471, 6472, 6473, 6474, and 6475 are shown in A-H respectively. Proximal is to the top of the image.

4.6.3 Haematology and serum inflammatory markers of scaffold implanted sheep

Haematology testing of all sheep for full blood count (including white cell count, neutrophil count, lymphocyte count, and red blood cell count) demonstrated no significant change from pre- to 3 months post-surgery levels. Serum markers for inflammation (serum amyloid A and fibrinogen) were not elevated at 3 months post-surgery compared to pre-surgery levels in all sheep (Table 4-4).

Table 4-4: Results of full blood count and serum inflammatory markers taken pre-surgery and pre-necropsy / 3 months post-surgery.

			Pre-surgery						3 months post-surgery					
Ref.	Age (years)	Weight (kg)	WBC (10e9/L) [Range 4-12]	Neut (10e9/L) [Range 0.7-6]	Lymph (10e9/L) [Range 2-9]	RBC (10e12/L) [Range 8-16]	Serum Amyloid A (mg/L)	Fibrinogen (g/L)	WBC (10e9/L) [Range 4-12]	Neut (10e9/L) [Range 0.7-6]	Lymph (10e9/L) [Range 2-9]	RBC (10e12/L) [Range 8-16]	Serum Amyloid A (mg/L)	Fibrinogen (g/L)
6468	6	87	6.30	1.01	4.60	8.38	0.0	3.5	6.65	2.19	3.59	9.37	0.0	3.2
6469	4	76	7.30	1.24	5.26	9.31	0.0	3.6	8.46	2.28	4.57	8.41	0.0	4.5
6470	6	67	5.90	1.18	3.95	10.59	0.0	3.0	6.50	1.95	4.23	10.42	0.0	3.4
6471	4	70	8.10	3.48	3.24	10.27	1.9	3.5	5.44	1.09	3.97	8.96	6.2	0.3
6472	4	76	6.60	1.25	4.88	12.04	0.0	3.2	6.74	1.28	4.38	10.21	0.0	2.8
6473	4	84	8.36	1.50	6.19	14.59	1.2	3.5	10.67	2.77	5.87	10.95	0.0	2.8
6474	6	69	**13.85**	**9.70**	3.32	9.72	0.0	5.0	11.12	4.23	5.56	10.74	0.0	3.2
6475	6	67	8.33	3.25	3.58	11.50	0.0	4.4	8.81	4.85	3.00	10.86	0.0	4.9

4.6.4 Histology of electrospun scaffold implanted tendons

Haematoxylin and eosin (H&E) staining demonstrated several key features. The electrospun scaffolds were present in an area of increased cellularity and vascularisation. Each section consistently demonstrated three distinct areas (Figure 4-4 A). The most superficial layer contained remnants of woven PDO monofilament. Residual polymer from the PCL layers were not consistently identified due to sectioning the tendon explants longitudinally, in the same plane as the PCL layers. Continuous and deep to this layer was the electrospun PDO layer. This area was occupied by a large number of cells and demonstrated neovascularisation. Remnant PDO material was rarely seen in this zone at high (100X) magnification. The deepest layer of the section represented the native sheep tendon underlying the patch. These areas had similar appearance to a normal tendon, but with fewer fibrils with the characteristic crimp morphology of normal tendon (Figure 4-4 D).

In the electrospun fibre zone, scant areas of FBGCs were rarely seen (Figure 4-4 F). A network of blood vessels supported the tissue integrated into the electrospun scaffolds (Figure 4-4 E). The neovascularisation observed was seen within the patch scaffold.

The electrospun (ES) fibre zones were significantly different in terms of fibroblast cell count (median 89, 95% CI 78-108 vs 16, CI 9-22, $p = 0.0002$), and

vascularity (median vascularity grade 2, CI 1.6-2.7 vs 0, p = 0.0002) compared to the normal tendon (NT) zones. There was no difference in FBGC count between the electrospun fibre and normal tendon zones. These cells were rarely seen in either zone (median 0 FBGCs seen in both ES and NT zones).

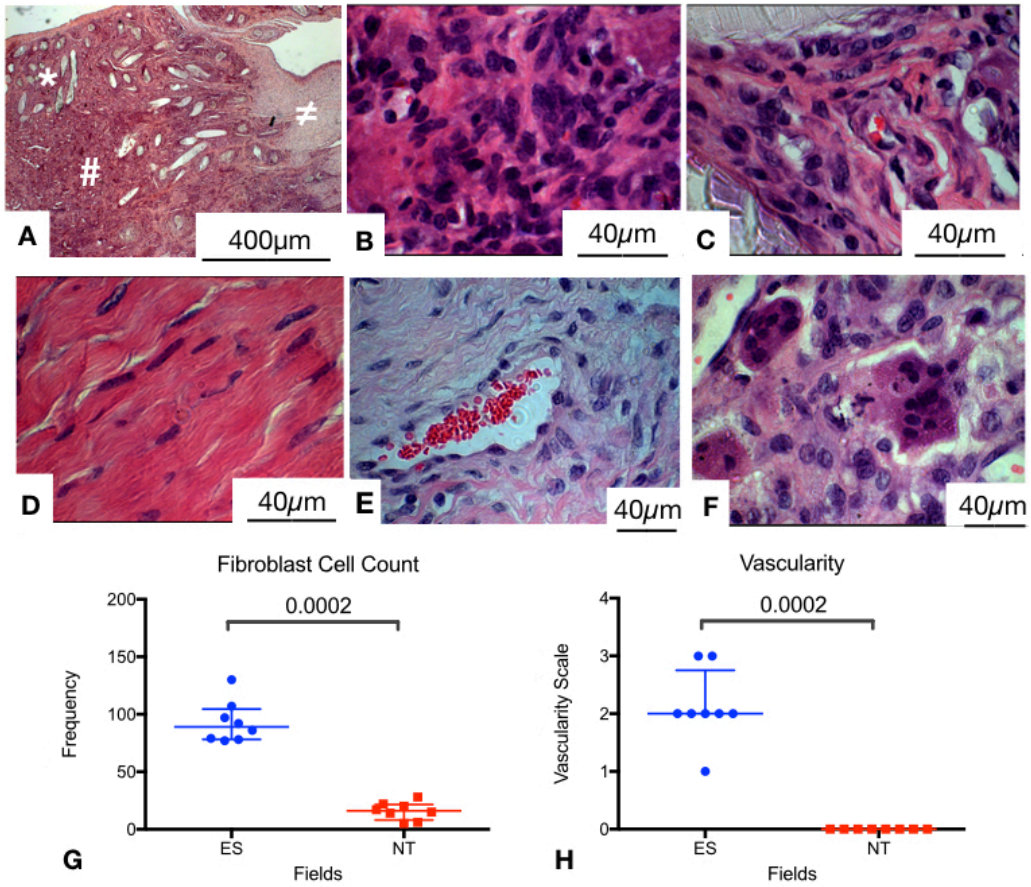


Figure 4-4 Histology of patch augmented ovine tendons. Figure A shows low magnification image illustrating woven PDO zone (*), electrospun PDO zone (#), and normal tendon zone (≠) of patch augmented repair. Different images represent different imaged fields including electrospun fibre zone (B), zone adjacent to woven PDO (C), and normal tendon zone (D). Figures E and F demonstrate blood vessel within electrospun zone and foreign body giant cell formation respectively. Quantitative analysis of fibroblast cell count comparing electrospun (ES) zone and normal tendon (NT) zone is shown in figure G. Vascularity grade is shown in figure H. P values (Mann Whitney U test) given above compared fields.

Table 4-5: Intra and inter-observer reliability for histology scoring system. Interclass coefficient values shown. Test, retest was performed by 2 independent blinded assessors, on two occasions 2 weeks apart.

Intra-observer	**Consistency**		**Absolute agreement**	
	Single measures	Average measures	Single measures	Average measures
FBCC	0.992	0.996	0.991	0.995
ICC	0.053	0.100	0.053	0.101
FGBCC	0.000	0.000	0.000	0.000
Vascularity	0.960	0.980	0.960	0.979

Inter-observer	**Consistency**		**Absolute agreement**	
	Single measures	Average measures	Single measures	Average measures
FBCC	0.995	0.998	0.995	0.998
ICC	0.741	0.851	0.744	0.853
FGBCC	0.661	0.796	0.661	0.796
Vascularity	0.964	0.981	0.960	0.979

4.6.5 Immunohistochemistry

There were significantly increased numbers of $CD4^+$, $CD45^+$, and $CD14^+$ cells in the electrospun fibre zones compared to the normal tendon zones ($p < 0.0001$, $p < 0.0001$, and $p = 0.0005$ respectively). However, fewer $CD14^+$ cells were identified than $CD4^+$ cells. TGFβ immunopositive staining was markedly increased in the electrospun fibre zones compared to the normal tendon zones ($p < 0.0001$) (Figure 4-5).

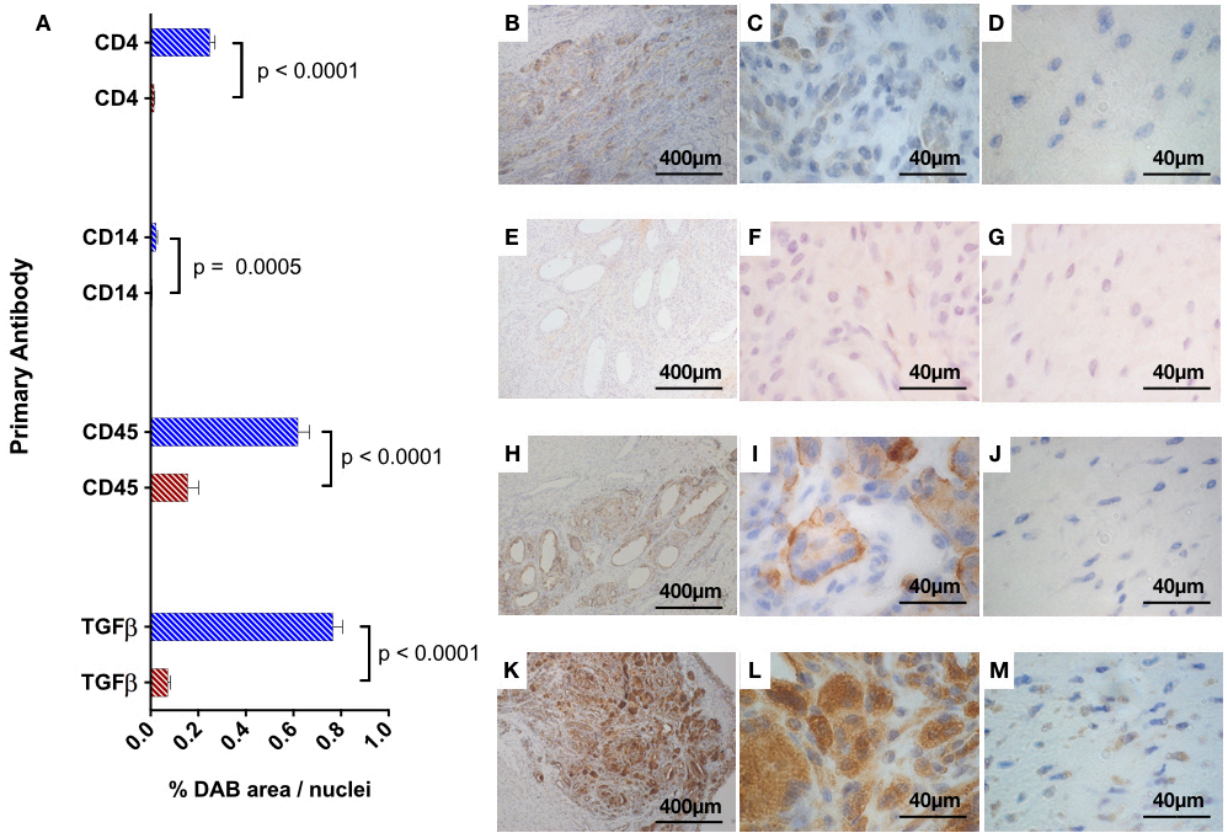


Figure 4-5. Immunohistochemical staining of patch augmented ovine tendons. (A) Quantitative immunohistochemistry (IHC) results for patch samples. Red bars = Normal Tendon (NT) zone. Blue bars = Electrospun fibre (ES) zone. Percentage area of DAB stain per nuclei for primary antibodies in zones imaged. P values (Mann Whitney U test) provided to the right of compared fields. Representative images showing immunohistochemistry (IHC) for anti-CD4, anti-CD14, anti-CD45, and anti-TGFβ. B/E/H/K: low magnification (10X) bright field images showing both ES and NT zones. C/F/I/L: images taken at high magnification (100X) in the electrospun fibre (ES) zones. D/G/J/M: images taken at high magnification (100X) in the normal tendon (NF) zones.

Given the high number of proliferative fibroblasts (characterised by their large nuclei, elongated and tapering cytoplasm at the poles of the nuclei), Collagen I and III staining was assessed. Collagen I α2 was observed within the normal tendon zones, however, more intense immunopositive staining was seen in the electrospun fibre zones. For Collagen III α1, no staining was present in the extracellular matrix of the normal tendon zones, however, the electrospun fibre zones showed immunopositive staining. The presence of Collagen III in the tissue surrounding the patch is consistent with fibrous tissue healing (Figure 4-6).

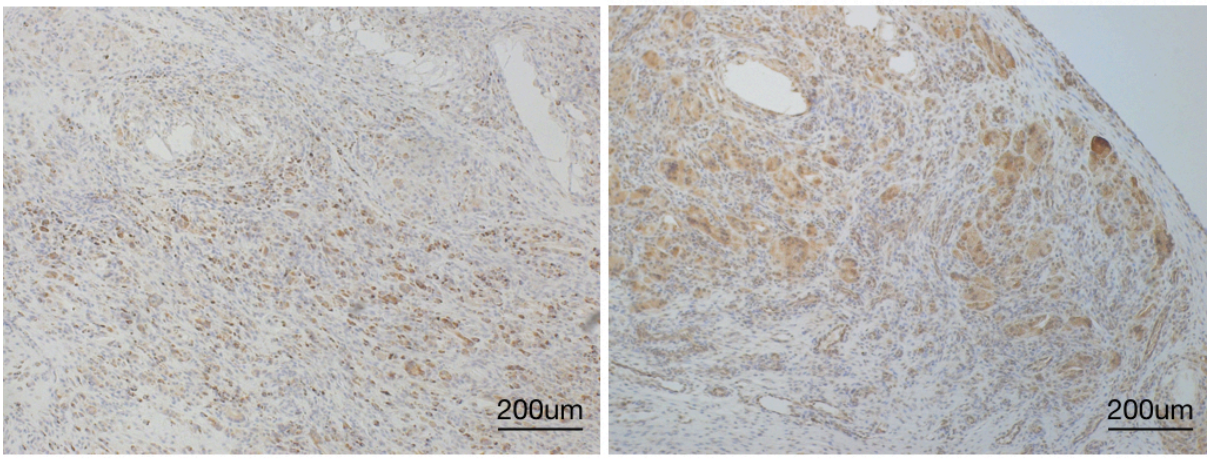


Figure 4-6: Immunohistochemical staining for collagen proteins in patch augmented tendon sections. Representative images showing immunohistochemistry (IHC) results for anti-collagen I (left) and anti-collagen III (right) in typical patch augmented tendon samples. Add detail for DAB immunopositive staining (brown) and nuclear counterstain (blue).

4.6.6 Adverse events

There were no adverse events noted. All sheep resumed normal activity after scaffold implantation. There was no evidence of lameness in any sheep after surgery.

4.7 Discussion

This *in vivo* study in an ovine non-healing model of tendon injury showed that implantation of an electrospun PDO/PCL scaffold promoted tendon healing. A mild local inflammatory response was seen 3 months after surgery, and was consistent with the expected host response to this biodegradable polymer. The electrospun component of the scaffolds integrated almost entirely with host tendon tissue in all sheep with minimal external signs of inflammation and without significant adhesion formation to the surrounding tissues. Some residual woven monofilament PDO was seen in 5 of 8 sheep at 3 months and demonstrated no cellular infiltration. The predominant cell type that infiltrated the electrospun PDO/PCL scaffolds were proliferative fibroblasts. This fibroblast infiltration response is in keeping with other studies, however it is limited by lack of available specific ovine tendon fibroblast markers to conclusively identify these cells. No systemic inflammatory response was detected by haematology and serology. There were no signs of infection or tumour formation at necropsy. Significant new blood vessel development was observed within the electrospun part of the scaffold and this accompanied the infiltration of proliferative fibroblasts. This is the first study to utilise this ovine model to test the safety and

efficacy of an electrospun polymeric patch for tendon augmentation. In addition, this patch was applied overlying the surgically-created defect only, without any sutures directly repairing the tendon. Despite this, the patch demonstrated a capacity to induce repair in a defect that has been shown to not heal spontaneously in this model. Other scaffolds have been tested in tendon repair, however they are not in an electrospun biodegradable polymeric patch structure. Additionally, this study combined macroscopic observations with haematological, serological, histological, and immunohistochemical analysis to describe the systemic, local, and cellular response to this novel electrospun patch.

4.7.1 Cellular infiltration into electrospun scaffolds

Several studies have implanted electrospun polymer scaffolds in various small and large animal models and shown responses in agreement with the work in this chapter[257-261]. These earlier studies have shown collagen-containing extracellular matrix production within the new tissue. Bhaskar et al used a twisted yarn of electrospun PCL fibres to repair a longitudinal defect created in the flexor digitorum longus tendon of C57/Bl mice[262]. They demonstrated migration of cells to the core of the scaffold within 3 days in the majority of samples. Other studies have shown significant fibroblast infiltration in various electrospun scaffolds[257 258 263 264]. Although not quantified, Manning et al reported no significant adverse reaction macroscopically, increased cellularity including increased fibroblasts, polymorphonuclear cells and monocytes in electrospun PLGA scaffolds implanted in canine flexor tendons[257]. At 120 days post Achilles

tendon repair using electrospun bovine collagen scaffolds in rabbits, Oryan et al showed the number of tenocytes, measured amongst the total cells within the defect, increased from 6% in the control group to 15% in the augmented group[258]. Implanting random and aligned electrospun PLLA scaffolds into two murine models, Yin et al demonstrated complete infiltration of spindle shaped cells along the nanofibres by 6 weeks[265]. One study contrarily found no increase in tenocyte density compared to conventional repair[263]. Buschmann et al implanted electrospun polyester urethane (PUR) / PCL tubes into rabbits' Achilles tendon defects, and showed no increase in collagen fibre density between those augmented with scaffold and those without[264].

4.7.2 Neovascularisation of electrospun scaffolds

This study demonstrates neovascularisation of collagen-containing tissue produced by proliferative fibroblasts in the area where the electrospun polymer scaffold had almost resorbed after 3 months *in vivo*. The observation of a rich network of new blood vessels has been a key finding in other *in vivo* applications of electrospun scaffolds. El-Fiqi et al implanted an electrospun polycaprolactone-gelatin composite in a rat bone defect model and demonstrated a thin fibrous capsule with neovascularisation of the scaffolds[266].

Several studies have successfully utilised electrospun PDO scaffolds as vascular grafts. Kalfa et al implanted electrospun PDO valved patches seeded with mesenchymal stem cells in lambs' right ventricular outflow tracts. Explants

showed no gross evidence of stenosis, aneurysm, or suture dehiscence. Macroscopically, they noted good tissue ingrowth, less fibrosis, less calcification, and no thrombus compared to control (pericardial patches). Histologically, the electrospun PDO scaffold had disappeared completely at 4 months, and was replaced with a three-layered vascularised tissue with collagen-containing extracellular matrix[260]. Pan et al implanted a PCL and a PCL-PDO composite electrospun scaffolds into the abdominal aorta of rats. A neointima formed after one month in the PCL-PDO scaffolds. With time, the intima matured and increased in thickness without causing stenosis. A similar phenomenon of endothelialisation with new blood vessel formation was seen on histology. Smooth muscle regeneration, assessed by immunocytochemistry with anti-α-SMA staining, was seen at 3 months[267]. Pontailler et al implanted various seeded and unseeded electrospun scaffolds of different polymer substrates including PDO, into the inferior vena cava of Wistar rats. All electrospun scaffolds showed patent grafts at 6 weeks on MRI. At 3 months, the PDO grafts (mean fibre diameter 578nm +/- 234nm) were fully resorbed and indistinguishable from the native IVC[261]. However, this neovascularisation is not always seen in all studies. Understanding the mechanisms that underpin this observation, and the influence of neovascularisation on healing, are a focus of future work by our group.

4.7.3 Foreign body giant cell response

Foreign body giant cells expressing CD45 are a common feature in *in vivo* studies of implanted electrospun polymer scaffolds[257 260 262], and are logically associated with scaffold resorption. Kalfa et al reported a transient increase in foreign body $CD45^+$ giant cells at 1 month that nearly completely resolved by 3 months[260]. The same pattern was seen in a previous study using this scaffold for infraspinatus tendon repair in Lewis rats[190]. The findings of this study represent one point in a timeline of a $CD45^+$ cell-predominated, inflammatory response. Given the single 12-week time point in this study, it is not possible to comment on temporal changes. The observation of cellular migration within the electrospun fibre zones of the patch is encouraging. The extracellular matrix produced by these infiltrating proliferative fibroblasts contains collagen I and III. Additionally, the cellular infiltration, ECM production and neovascularisation of the patch electrospun zone shows that scaffolds for tendon tissue engineering do not require the additional growth factors to induce tendon healing in this model.

4.7.4 Immune cell response to electrospun scaffolds

In this study, scant immune cells were seen in all sheep. Of the immune cells present, the largest proportion were $CD45^+$ cells. Immunopositive staining for CD4 was also increased compared to the normal tendon zones. $CD14^+$ cells, were significantly increased in the electrospun fibre zones compared to the normal tendon zones, however they were detected in much smaller levels than

$CD45^+$ and $CD4^+$ cells. It is imperative that the right cell populations are present at the various stages after tendon injury to instruct optimal repair mechanisms through modulating inflammation, driving controlled output of extracellular matrix, and potentiating resolution of inflammation. Some immune cells, as seen in this study, are required to degrade the polymer material as well as stimulate resident tendon stromal cells to produce extracellular matrix components[247]. In the Bhaskar et al study, using a twisted yarn of electrospun PCL fibres, they found an increase in CD45 expression compared to autograft control, although this was not quantified[262]. By contrast, Buschman et al, using an electrospun Polyurethane / Polycaprolactone (PUR/PCL) scaffold in rabbits, identified no macrophages at 3 months. The expression of $CD45^+$ cells has been found to change over time after implantation of electrospun scaffolds and these differences may reflect temporal changes in immune cell populations and profiles[264].

4.7.5 Strengths and limitations

The strengths of this study are firstly that it supports our previously published results in a rat model of infraspinatus tendon injury[190]. Secondly, a large animal non-healing model of tendon injury to assess efficacy was used. There are no other validated small or large animal models of tendon injury where the injury consistently fails to heal without intervention. However, there are also several limitations to this study. First, the single time point used for tendon harvest does not allow for a longitudinal assessment of scaffold-tendon interaction. Second,

there is a lack of reliable and specific cell markers for tendon fibroblasts. Hence, there is a reliance on identification using cell histomorphometry. The most commonly used cell markers for tendon fibroblasts, are also found to be expressed in various immune cells, therefore it is currently impossible to accurately identify these cells on immunohistochemistry. Third, it is uncertain whether the neovascularisation seen in the electrospun fibre zones is a positive or negative indicator of tendon healing. Normal tendon tissue is often lacking in extensive vasculature and little is known about the role of these new blood vessels observed within tissue integrated into this electrospun patch. Fourth, an internal normal tendon control for direct comparison was used. This study did not assess tissue response to woven PDO alone. A limitation of sectioning the tendon explants longitudinally, and not transversely, meant it was not possible to consistently identify the PCL layers sandwiched between the electrospun PDO layers. Transverse sections of tendon samples are technically difficult to cut using a rotary microtome, often disrupting the matrix and precluding histological and immunohistochemical analysis. Hence, it is not possible to decisively examine the cellular reaction to the PCL layer specifically. Finally, the explanted tendons were not tested for biomechanical properties such as stiffness and load to failure. However, the longitudinal defect is unlikely to significantly alter the tendon strength as it sections relatively few tendon fibres.

Future work will focus on further identifying the mechanism for new blood vessel growth in electrospun PDO scaffolds, long term evaluation of changes occurring

in new extracellular matrix deposition within scaffolds, and an attempt to characterise the biomechanical properties of augmented tendons. Additionally, electrospun scaffolds can be applied to augment restoration of tendon-bone entheses. There are few reliable, validated, *in vivo* enthesis repair models, however a natural next step would be to investigate electrospun scaffolds influence on restoring this tendon to bone interface. Finally, these results support the translation of electrospun scaffolds for tendon repair into first-in-man clinical safety trials.

4.8 Conclusions

A novel laminated electrospun and woven PDO and PCL polymeric scaffold induced tendon repair in a non-healing ovine surgical model of tendon injury. The scaffolds did not cause a systemic inflammatory response. They did induce a limited local inflammatory response consistent with repair but not an abnormal or excessive foreign body response. The local tissue response was characterised by increased vascularisation, fibroblast infiltration, moderate infiltration of $CD45^+$ and $CD4^+$ immune cells, and few foreign body giant cells. Future studies are planned to investigate the safety and efficacy of these scaffolds to augment tendon repair in humans.

4.9 Clinical implications of electrospun PDO/PCL patch for rotator cuff augmentation

The findings of the *in vivo* study presented in this chapter are encouraging for a potential augmentation strategy to improve the healing rate of human tendon tears. Many implants have been licensed for clinical use with less scientific evidence as to their mechanism, safety, and efficacy. However, careful application of these electrospun PDO/PCL patches in humans, under controlled circumstances, with sufficient monitoring, is required before widespread advocacy of their use. The results of the work presented in this chapter, and preceding experiments published in the scientific literature must be interpreted in context, and with consideration of their limitations. Firstly, no animal model adequately replicates the human disease. Rotator cuff repairs in human can have a preceding acute traumatic event, whilst most do not. Many patients undergoing surgery will have pain and functional limitation for several months before surgical intervention. The mean age for rotator cuff repair in the UK is 60 years old, whilst the animals used here are considered mature, but cannot be directly compared to a human cohort in terms of equivalent age. Additionally, the *in vivo* experiments presented in this chapter have shown efficacy in tendon healing, but did not involve a tendon-to-bone reconstruction. However, this patch was almost completely degraded by 3 months, and did not produce a systemic response, nor an adverse local immune response. Following appropriate evaluation in a first-in-man trial, electrospun PDO/PCL patches may become a feature of the repair construct in the near future.

The electrospun PDO/PCL patch for rotator cuff augmentation may prove to be effective at improving the healing rate however, there are limitations in its widespread adoption. Firstly, the application of a patch as an overlay to a repair will add time to the surgery. If performed arthroscopically, it may be challenging to pass the patch through the portals and secure it using extracorporeal knot-tying. In both the open and arthroscopic techniques, there will be a learning curve to overcome. Finally, whilst some surgeons use patch augmentation in select cases, it remains relatively infrequently used. Hence, shifting surgical decision-making to include electrospun patch augmentation in all patients undergoing repair will be difficult. However, if the electrospinning principle can be applied to a component of the repair construct that is used in every case, adoption may be easier to achieve. The next section will consider the rotator cuff repair construct and the innovation in sutures used for rotator cuff repair.

4.10 The evolving rotator cuff repair construct

Rotator cuff repair surgery has evolved over the past few decades. Initial constructs for repair were simple sutures grasping the tendon edge, passed through bone tunnels drilled through the greater tuberosity, and tied in a knot to secure them. As implant technology evolved, suture anchors came into popular use. Suture anchors come in different materials, with varying deployment mechanisms, and with numerous options of preloaded sutures. Metal anchors gave way to more popular PEEK or biocomposite anchors, however, there are

many options available to surgeons today. Sutures loaded into these anchors, are mostly strong, braided, multifilament, and synthetic. As arthroscopic instruments improved, so did our understanding of cuff tear types. In time, new techniques were developed, in accompaniment with innovation in implants. Modern techniques however, still rely on suture anchors, and sutures loaded into these anchors. Sutures are currently employed in all rotator cuff repairs and are therefore an ideal target for application of electrospinning to produce bioactive sutures that stimulate tendon healing.

4.10.1 Innovation in sutures

Implant companies that produce suture anchors for rotator cuff repair will offer several suture options preloaded into them. Furthermore, surgeons may wish to use a different suture type and many anchors will accommodate additional sutures. Most sutures are very strong, multifilament, braided, and non-degradable. Unlike anchors, suture innovation has not been a key feature of the evolution of rotator cuff repair surgery. Sutures are used in every rotator cuff repair. Using electrospinning, multifilament sutures can be made to a specific design and application. Electrospinning allows for a number of options, with varying number of filaments, bundles, and a variety of polymer solutions. These electrospun filaments can be used to create a twisted or braided suture. Electrospun filaments may also be combined with traditional extruded sutures to form a composite suture with desired mechanical properties. The next chapter will focus on investigating a novel electrospun suture compared to a traditional

monofilament suture of the same polymer substrate in an animal model, similar to that used in this chapter.

Chapter 5: Multifilament biodegradable electrospun sutures

The previous chapter investigated the safety and efficacy of an electrospun PDO/PCL patch to augment tendon repair using a non-healing ovine model of injury. The results demonstrated healing in all sheep at 3 months after implantation, with significant fibroblast infiltration and neovascularisation within the scaffold. In this chapter a multifilament electrospun PDO suture will be investigated using the same model. These bioactive electrospun PDO sutures have been designed to be used instead of conventional sutures in tendon repair. One particular application is in rotator cuff repair, whereby modern constructs have seen an evolution in technique and implants, but healing remains a challenge.

5.1 Rotator cuff repair construct

For surgical repair of torn rotator cuff tendons, surgeons frequently utilise a combination of suture anchors, preloaded with sutures that are passed through the tendon. These can either be tied down (knotted repair), or used to hold the tendon in place without the need for knots (knotless repair)[69]. A “double row” technique may be preferable whereby the medial part of the tendon is attached with suture anchors to the medial part of the footprint and the free tendon edge is attached to the lateral part of the footprint using a second lateral row of anchors[268]. This double row technique can be linked to the medial row or unlinked, knotted or knotless, or a combination (Figure 5-1)[69]. A trans-osseous equivalent technique using suture anchors has also been described. This is

similar to a double row repair but with placement of the lateral row in a more lateral position[269].

This evolution in surgical techniques and suture anchor technology has been accompanied by innovation in sutures used in rotator cuff repair. Generally, sutures used in rotator cuff repair are non-absorbable, braided, and much stronger than the native tendon[270 271]. Recent innovations include the development of suture tape, with a wider surface area, to better compress the tendon to the bony footprint[197]. Despite the aforementioned advances in techniques and implants, many of which have shown superior mechanical properties in a laboratory setting[66 85 196 217], clinically significant improvements to patient reported outcomes, or superior radiological healing rates, have not been demonstrated[32 139 272].

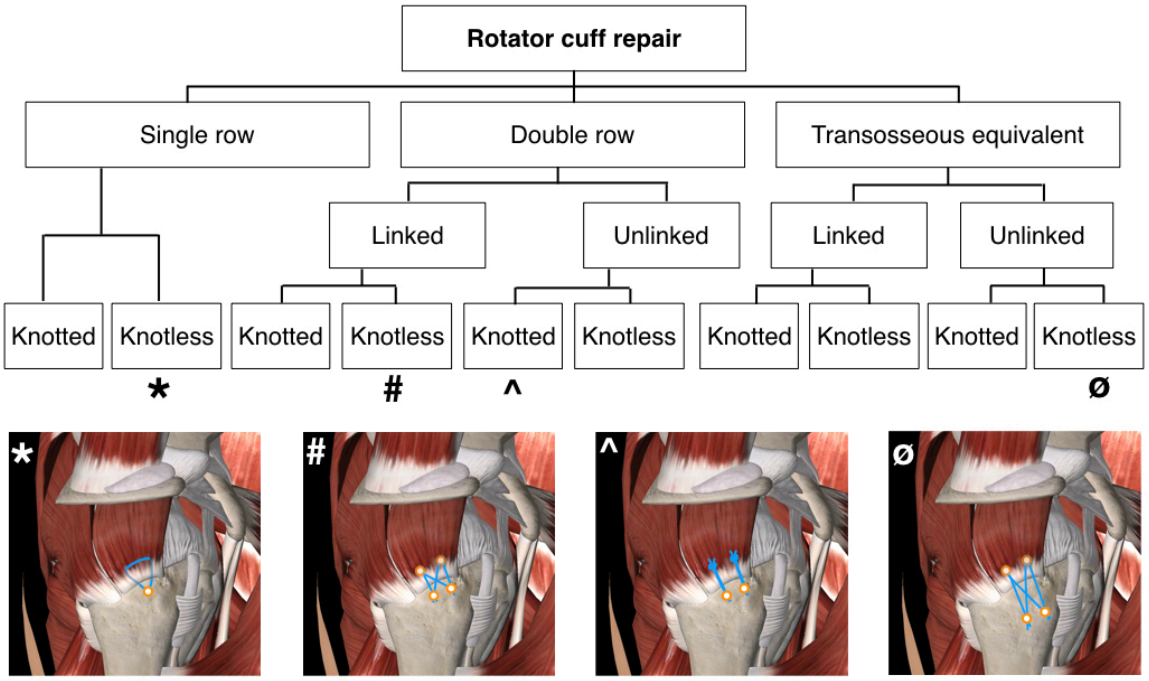


Figure 5-1: Rotator cuff repair construct options. Various construct options for rotator cuff repair including number of rows, linked vs unlinked rows, and knotted vs knotless techniques. Four example constructs are shown below. Note that some constructs may include a knotted medial row and a knotless lateral row. This is a common construct type as added construct security with the knots may be achieved, as well as increased medial row compression.

5.1.1 Failure following rotator cuff repair

The location of failure following cuff repair can be at the anchor-bone interface, the tendon-suture interface (Type 1 failure), or from a recurrent tear, medial to the original repair (Type 2 failure)[273 274]. The commonest mode of failure is from 'cheese wiring' of the suture through the repaired tendon. Whilst this has been reduced in biomechanical studies by using braided sutures to increase the coefficient of friction[85 197 275], the native tendon remains the vulnerable link.

Currently, sutures used in rotator cuff repair are inert structures of synthetic material. They confer no favourable advantages to the native tendon other than mechanical ones, helping transfer load from the muscle to the bone, and providing compression of the healing tendon to the bone. In light of the results of the previous chapter, it is thought that bioactive sutures, providing the native tendon with biophysical cues, may positively influence healing.

5.2 Development of electrospun PDO sutures

To achieve the transition from an inert structure to a bioactive material, microscale filaments that mimic the native extracellular matrix structure, have been proposed[191]. Our group has developed a method to produce continuous electrospun (ES) polydioxanone (PDO) filaments, which can then be assembled into multifilament yarns serving as a suture[191]. In previous work, a favourable cellular response to these electrospun sutures has been demonstrated *in vitro*[191]. Following implantation in a rodent infraspinatus repair model, this electrospun suture showed cellular infiltration and a transient foreign body giant cell (FBGC) response, similar to that seen in the electrospun patch of the previous chapter[191]. Polydioxanone has been used in surgery as a suture material since 1982[276]. It is a biodegradable monofilament suture that can be applied in a wide range of surgical procedures. It is not currently used as definitive fixation in rotator cuff repair, as stronger, non-biodegradable, braided sutures have become popular. This monofilament PDO suture, called PDS, is thought to be relatively inert *in vivo*[277]. Similar to the electrospun patch, a multifilament electrospun PDO twisted yarn was designed for strategic

biomimicry, in order to convert an inert material (PDO) into a bioactive suture. The *in vivo* tissue response in tendon repair using an ovine model will be determined in this chapter.

5.3 Aims

The aims of this study were to investigate, in a validated large animal (ovine) non-healing tendon defect model, whether electrospun PDO sutures:

1. induce tendon repair.
2. are safe to use without producing a significant systemic or local foreign body tissue response.
3. promote cellular infiltration compared to a PDO monofilament suture (control).

5.3.1 Hypotheses

It was hypothesised that:

1. Electrospun PDO sutures promote healing in tendon repair.
2. Electrospun PDO sutures do not cause a systemic response or an adverse local immune reaction.
3. Electrospun PDO sutures allow for significantly more cellular infiltration than PDO monofilament sutures.

5.4 Materials and methods

5.4.1 Suture manufacture and characterisation

These ES sutures have been developed prior to the start of this DPhil and were fabricated by Antonina Lach* using a method previously published[191]. Each step is described in sections 5.4.2 - 5.4.5.

5.4.2 Preparation of the electrospinning solution

Polydioxanone (PDO, 1.5–2.2 dl/g, Evonik, Essen, Germany) was dissolved into 1,1,1,3,3,3-hexafluoroisopropanol (HFIP 99.9% purity, Halocarbon Products Corporation, Atlanta, GA, USA) at concentrations of 11% (weight to volume ratio). The solutions were agitated at room temperature on a roller for at least 24 hours prior to use to allow for complete dissolution of the polymer granules.

5.4.3 Electrospinning filaments

Electrospinning was performed in an enclosed container using a single nozzle high voltage power supply system (30 kV, SL30P30/230, Spellman, West Sussex, UK) and a syringe pump (World Precision Instruments Limited, Florida, USA). Filaments were produced by using a thin stainless steel wire (100µm in diameter, Goodfellow, Huntingdon, UK) as a collector. The wire was moved underneath the nozzle at a speed of 0.5 mm/s with a bespoke winding unit. The distance between the nozzle and the wire was 20cm, and the average voltage

*Antonina Lach is a member of the Carr research group and is a biomanufacturing technician with expertise in electrospinning scaffolds. (https://www.ndorms.ox.ac.uk/team/antonina-lach)

applied was 6.5 - 7.3kV. Electrospun filaments were then continuously wound up onto a motorised filament spool, separating them from the wire collector.

5.4.4 Filament drawing and multifilament assembly

Collected electrospun filaments were drawn manually to produce stretched filaments. Stretched filaments were then twisted into a ply yarn in the “S” direction (3 or 5 filaments per ply). Seven ply yarns were then twisted in the “Z” direction to produce assembled cord yarns. A twist ratio of 2:1 (S:Z) turns per metre was maintained throughout all samples.

5.4.5 Annealing and sterilisation

Cord yarns were annealed at 65^{o}C for 3 hours to produce the ES suture. All samples were stored in a desiccator at room temperature until implantation. Prior to implantation, sutures were sterilised in 70% ethanol solution for 6 hours.

5.4.6 Scanning electron microscopy (SEM)

Samples were analysed, after gold coating using a SC7620 Mini Sputter Coater System (Quorum Technologies Ltd, East Sussex, UK), by scanning electron microscopy (SEM) using a Zeiss Evo LS15 Variable Pressure Scanning Electron Microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). 3 images were taken at 5000X magnification to allow a minimum of 30 fibres to be measured for their diameter.

5.4.7 Surgical procedures

The study was performed under UK Home Office license (PPL 70/6105) and with approval from the Ethics and Welfare Committee of the Royal Veterinary College (RVC). In a validated model[253], 8 female, skeletally mature, English mule sheep underwent open surgery of the digital sheath. Using a 2mm hook knife (ECTRA II disposable triangle knife, Smith and Nephew, UK), introduced through a proximal portal, a longitudinal surface defect (10mm long; 2mm deep) was created in the deep digital flexor tendon (DDFT) within the digital sheath, palmar to the metacarpophalangeal (MCPJ) joint in the right forelimb (Figure 5-2). The surgical defect was created in the coronal plane of the lateral branch of the DDFT only, which has been shown to not heal spontaneously over a 6 month period[253]. All surgeries were performed under general anaesthesia induced with xylazine, ketamine, and midazolam. Anaesthesia was maintained with ~2% isoflurane gas. The tendon defect was repaired with a 3-0 monofilament PDO (control) suture (MF) and a PDO electrospun suture (ES) using a simple surgeon's knot in eight sheep. At the same time, the palmar annular ligament was transected to allow more space for the suture knots to move within the fetlock canal.

5.4.8 Necropsy and macroscopic observations

At 3 months, all sheep were euthanised by overdose of pentobarbitone at a dose of 1ml/kg body mass. Necropsy was performed in all sheep according to standard protocols. During tendon harvest, all repairs were examined macroscopically for signs of tissue healing, residual inflammation, knot retention,

synovial fluid changes, and adhesion formation. The general appearance of the surrounding DDFT sheath and adjacent superficial digital flexor tendon (SDFT) was also recorded. Circumferential measurements proximal and distal to the metacarpophalangeal joint (MCPJ) were made to assess swelling of the tendon repair site.

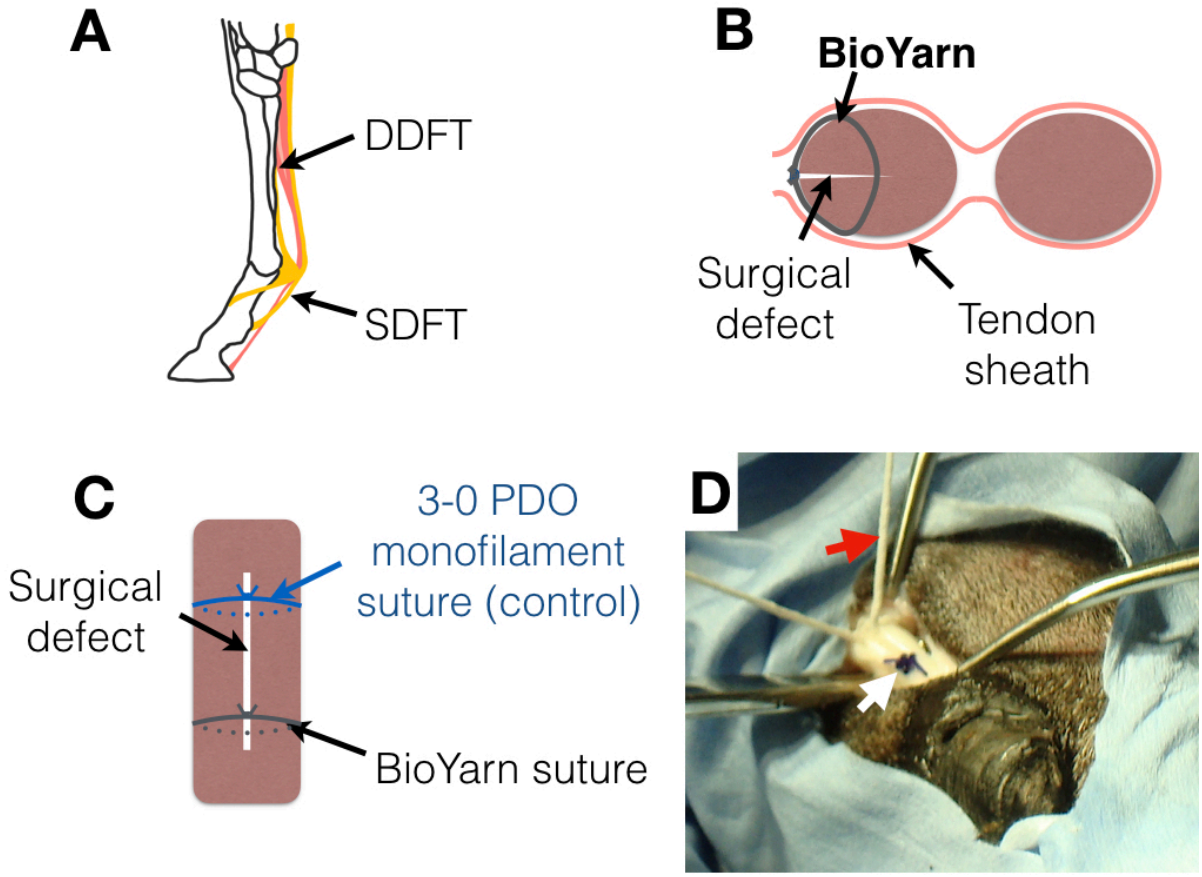


Figure 5-2 Implantation of electrospun and monofilament PDO sutures to augment tendon repair in an ovine model. A) Diagram representing side lateral aspect of the ovine forelimb, illustrating the relationship between the deep digital flexor tendon (DDFT) and the superficial digital flexor tendon. B) Schematic showing the transverse section of the deep digital flexor tendon (DDFT) repair model. C) Schematic showing lateral view of the defect in the DDFT repaired with the MF monofilament (control) suture and the electrospun yarn scaffold suture. D) Digital photograph showing surgical repair of the DDFT defect showing ES suture (red arrow) and MF monofilament control suture (white arrow).

5.4.9 Haematology and serum inflammatory markers

Blood samples for haematology testing were taken to rule out a systemic, infective or inflammatory response. Samples were taken from all sheep pre-surgery and 3 months post-surgery. Full blood count and serum inflammatory markers (serum amyloid A and fibrinogen) were performed by the clinical pathology laboratory at the Royal Veterinary College (RVC).

5.4.10 Histology

The operated lateral branch of the harvested DDFT was cut at the bifurcation and fixed in 10% formalin for 7 days. Tendon samples were then processed using a Leica ASP300S tissue processor and embedded in paraffin wax. Using a rotary RM-2135 microtome (Leica Microsystems Ltd, Milton Keynes, UK), 5-7µm sections were cut and placed onto Surgipath X-tra Adhesive glass slides (Leica Microsystems Ltd, Milton Keynes, UK) and stained with haematoxylin & eosin (H&E). Using a Zeiss AX10 inverted microscope with an Axiom HRc camera and Axiovision software (Zeiss, Cambridge, UK), bright field microscopy images were taken at high (100X) magnification in several fields corresponding to areas relating to the electrospun fibre zone (ES), tissue adjacent to the control monofilament suture zone (MF), and normal tendon zone (NT).

Taking 6 random images per field, per sample, histomorphometric features were used to quantify immune cell count (ICC), fibroblast cell count (FCC), and foreign body giant cell count (FBGCC). Vascularity was evaluated semi-objectively using the following grading system:

0 = no signs of vascularity;

1 = occasional red blood cells (RBCs) seen, or one small vessel;

2 = several groups of RBCs, or more than one small blood vessel;

3 = extensive blood vessels, one or more large vessel(s), or many RBCs seen in the field of view.

5.4.11 Statistical analysis

Descriptive statistics included median values and 95% confidence intervals (CI). Comparisons between zones (ES, MF, or NT), using median values for immune cell count, fibroblast cell count, foreign body giant cell count, and vascularity were performed. A Mann Whitney U test was applied to determine the differences between fields (NF, MF, and NT) in the 4 categories (ICC, FCC, FBGCC, and vascularity) using Prism v7 (GraphPad, La Jolla, CA, USA). The level of statistical significance was set at <0.05.

5.5 Results

5.5.1 Electrospun (ES) suture characterisation

Suture scaffold characterisation using scanning electron microscopy revealed the twisted multifilament structure (Figure 5-3 D), with electrospun microfibres aligned in the direction of the suture. The median fibre diameter was 1.05µm (IQR 0.76 – 1.31µm). Micro computed tomography (MicroCT) images of the electrospun suture scaffold in cross section is shown in Figure 5-3 G, illustrating the potential spaces between multifilament bundles for cellular infiltration. In

comparison, the monofilament suture (MF), seen in Figure 5-3 I, is a solid cylindrical polymer suture in cross section.

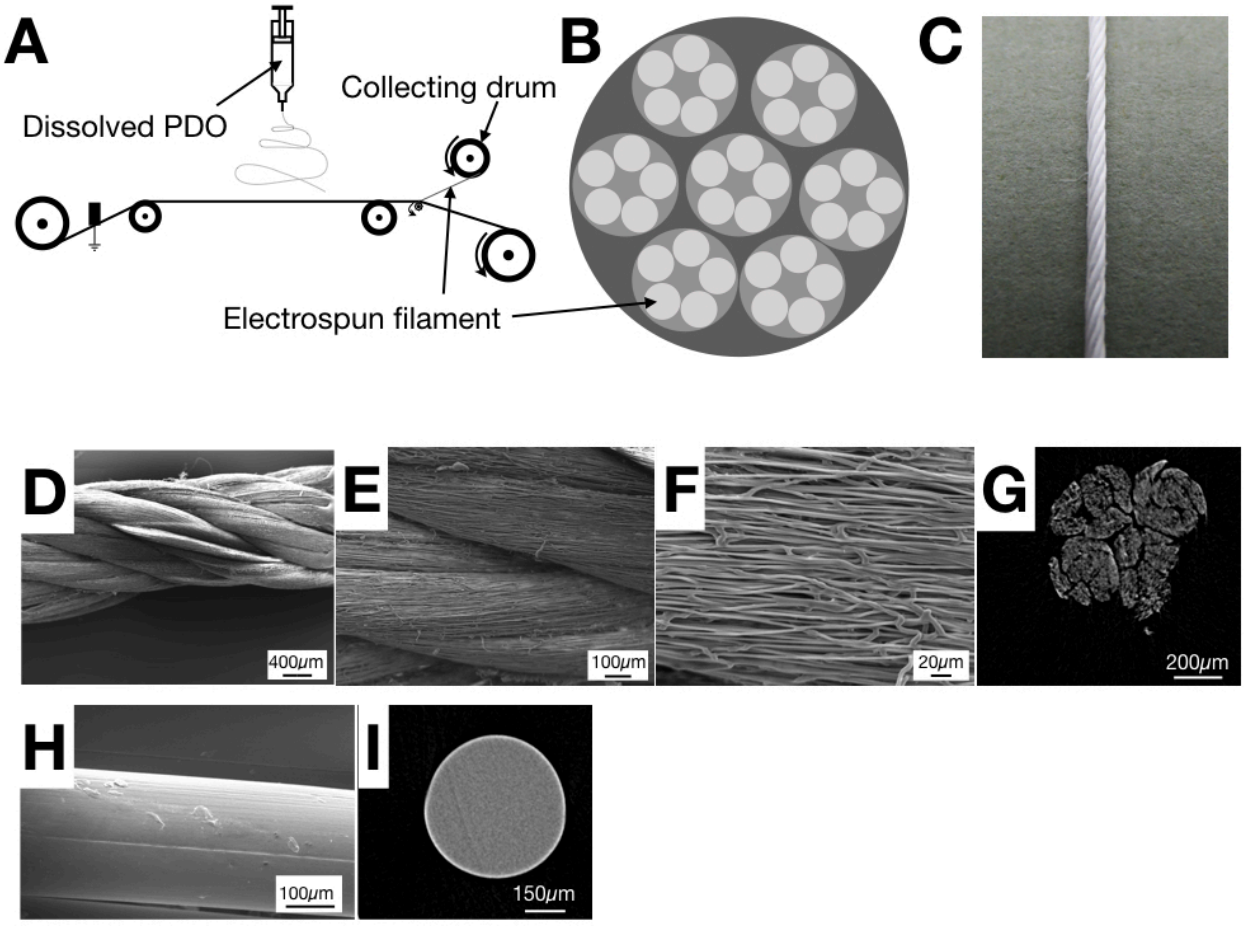


Figure 5-3 Production and characterisation of electrospun PDO suture. A) illustrating the setup for producing electrospun (ES) filaments. B) Diagram of the ES suture structure. C) Photograph showing the electrospun suture. D-F) Scanning electron microscopy (SEM) images showing twisted multifilament bundles of electrospun fibres at different magnifications (D-F). G) MicroCT image of cross section of electrospun multifilament twisted suture prototype. Note the potential spaces between filaments for cellular infiltration. H) SEM image of monofilament suture (MF). I) MicroCT image showing cross section of monofilament suture (MF).

5.5.2 Macroscopic observations

None of the sheep showed signs of infection or tumour at necropsy. There was no difference in the circumference of the forelimb around the repair site (above and below the ergot) measured pre-surgery and 3 months post-surgery (Table 5-1). Macroscopic inspection of the lateral branch of the deep digital flexor tendon demonstrated an absence of any surgically induced tendon defect in all sheep, 3 months after the ES and MF suture implantation. There was no residual polymer from either suture seen in any specimens on inspection.

Table 5-1 Results of circumference measurements (in cm) of the right forelimb of the sheep taken above and below the ergot pre-surgery and pre-necropsy / 3 months post-surgery.

	Pre-surgery		3 months post-surgery	
Ref.	MCPJ circumference - Proximal (cm)	MCPJ circumference - Distal (cm)	MCPJ circumference - Proximal (cm)	MCPJ circumference - Distal (cm)
6459	11.0	10.5	12.0	11.5
6462	12.0	11.0	12.0	10.5
6463	12.0	11.5	12.0	13.5

6464	12.0	11.0	12.0	13.0
6465	12.0	11.0	12.0	11.5
6466	10.0	10.5	12.0	12.0
6496	11.5	11.0	11.0	11.5
6497	10.5	10.5	12.0	11.0

The contour of the healed tendon over the defect was altered. A smooth yet irregular lateral border with hyperaemia was observed in all specimens. Two sheep formed a fibrous capsule around a retained surgical knot from the ES suture (Figure 5-4 D/E). All sheep demonstrated a normal amount (<0.5ml) of synovial fluid with normal appearance, mild or no adhesion to the sheath, and a mild local inflammatory reaction.

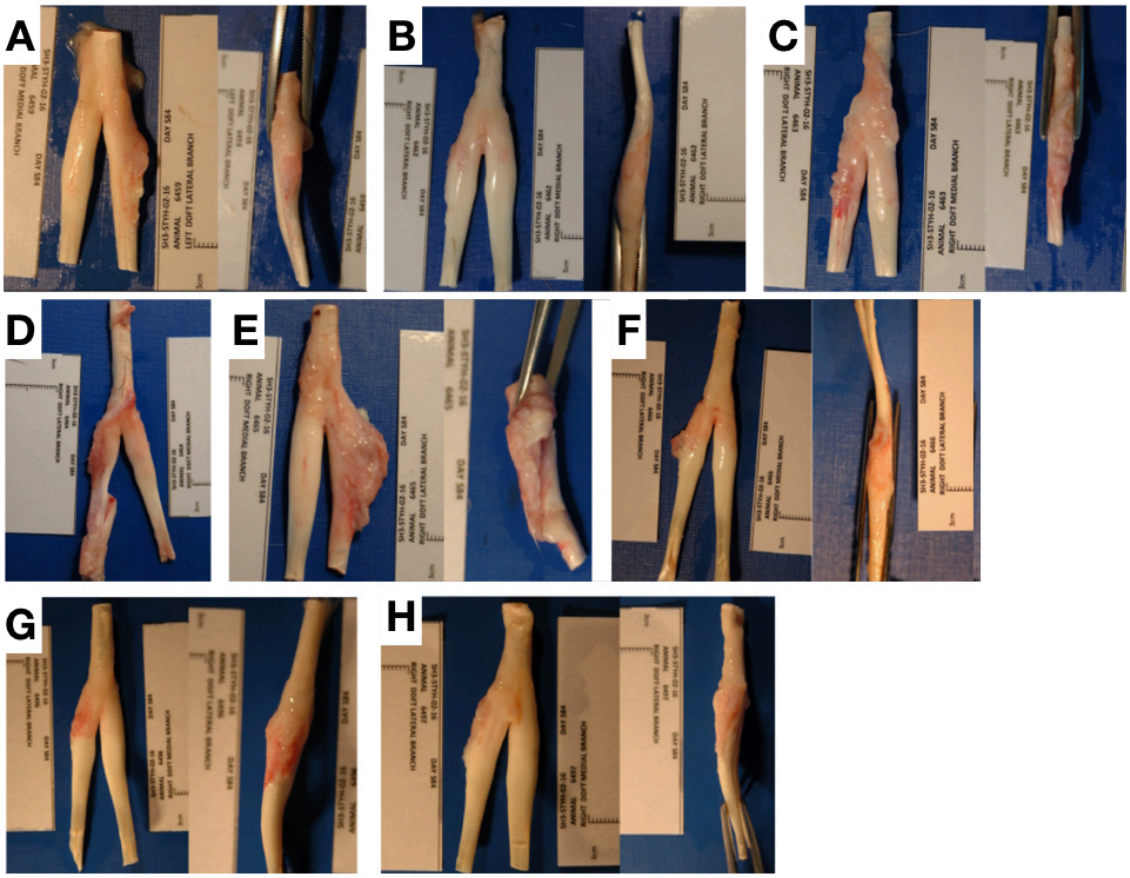


Figure 5-4 Macroscopic appearance of repaired ovine tendons using electrospun PDO suture and extruded monofilament PDO suture. Digital photograph images showing harvested tendons augmented with the ES sutures at 3 months post repair. Sheep 6459, 6462, 6463, 6464, 6465, 6466, 6496, and 6597 are shown in A-H respectively. Proximal is to the top of the image.

5.5.3 Haematology and serum inflammatory markers

Haematology testing of all sheep for full blood count (including white cell count, neutrophil count, lymphocyte count, and red blood cell count) demonstrated no significant change at 3 months post repair. Serum markers for inflammation

(serum amyloid A and fibrinogen) were not elevated in all sheep at 3 months post-surgery compared to pre-surgery levels (Table 5-2).

Table 5-2 Results of full blood count and serum inflammatory markers taken pre-surgery and pre-necropsy / 3 months post-surgery.

	Pre-surgery								3 months post-surgery					
Ref.	Age (years)	Weight (kg)	WBC (10e9/L) [Range 4-12]	Neut (10e9/L) [Range 0.7-6]	Lymph (10e9/L) [Range 2-9]	RBC (10e12/L) [Range 8-16]	Serum Amyloid A (mg/L)	Fibrinogen (g/L)	WBC (10e9/L) [Range 4-12]	Neut (10e9/L) [Range 0.7-6]	Lymph (10e9/L) [Range 2-9]	RBC (10e12/L) [Range 8-16]	Serum Amyloid A (mg/L)	Fibrinogen (g/L)
6459	2	78	N/A	N/A	N/A	N/A	0.7	N/A	5.90	2.24	3.13	12.64	0.0	2.3
6462	3	90	5.16	1.96	2.84	10.78	0.0	3.2	6.16	1.54	3.76	9.53	1.7	3.2
6463	3	80	6.21	1.99	3.79	12.58	0.9	3.4	5.99	2.16	3.47	11.25	0.6	3.4
6464	3	90	6.20	2.11	3.60	12.19	0.0	3.2	6.59	1.98	3.36	11.13	0.8	4.1
6465	3	72	6.04	1.57	3.81	9.70	0.5	2.6	6.12	1.65	3.92	8.94	0.0	2.5
6466	6	71	7.13	1.50	5.35	10.88	0.0	3.3	7.86	1.97	5.34	9.67	0.0	5.1
6496	6	71	4.76	1.67	2.76	11.81	0.0	3.6	8.50	3.57	4.17	10.30	1.3	7.0
6497	6	56	5.97	2.45	2.21	11.87	0.0	3.6	7.42	3.12	3.12	8.96	2.2	2.7

5.5.4 Histology results

Haematoxylin and eosin (H&E) staining demonstrated several key features. The electrospun sutures represented an area of highly cellular, vascularised tissue. The monofilament (MF) suture (control) was visible in 3 of 8 specimens, and represented a defect devoid of any cells and without any increase in vascularity. The other 5 specimens demonstrated areas where the monofilament suture was extracted from the sample during sectioning with the microtome (Figure 5-5 D). This represents a lack of tissue integration into the monofilament. Some mild change to the tissue immediately adjacent to this monofilament suture was observed, specifically an increase in fibroblast cell count. This zone was surrounded by an area of tissue with a normal-like tendon appearance, characterised by few, well-aligned fibroblasts within a crimped extracellular matrix.

In both the ES and MF suture zones, foreign body giant cells were rarely, if at all, seen. A network of blood vessels supported the tissue integrated into the ES suture only. The neovascularisation observed was seen in the electrospun zone (ES) but not the tissue adjacent to the MF control suture, or the normal tendon zone (NT).

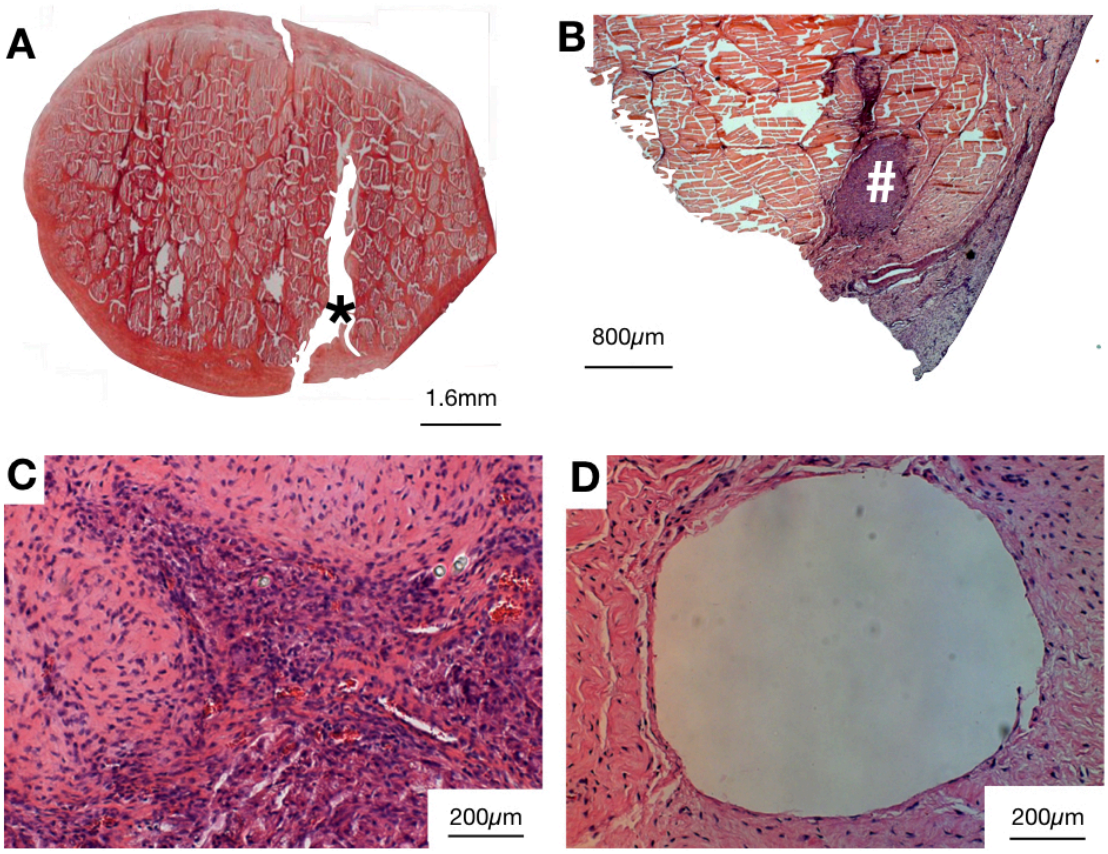


Figure 5-5 Histological appearance of ovine electrospun suture implanted tendons. A) Representative histology image (H&E stain) of cross section of DDFT tendon injury, 3 months after surgically-induced injury, showing absence of healing (*). B) Histology (H&E stain) image of cross section of DDFT, absence of defect and presence of the ES suture (#). C) Histology image (H&E stain) showing fibroblast cells infiltrating the ES suture and accompanied by blood vessels. D) Histology image (H&E stain) showing defect in tendon left by the control suture (extracted by microtome), without cellular infiltration or blood vessels.

Quantitative analysis showed that the ES suture zone was more vascular than the normal tendon zone and the MF (control) zone (median vascularity grade ES

2.3 (CI 1.5-3.0) vs MF 0.6 (CI 0.2-1.1) vs NT 0 (CI 0, $p < 0.00001$ and $p = 0.09$, respectively). The median number of fibroblasts in the ES suture zone (103, 95% CI 81-122) was significantly greater than in the control (32, 95% CI 15-55) and normal tendon zones (13, 95% CI 9-20, $p = 0.002$ and 0.001 respectively). There was no difference in the median FBGC count and immune cell count in all three zones (Median 0, FBGCs seen in ES, MF, and NT zones).

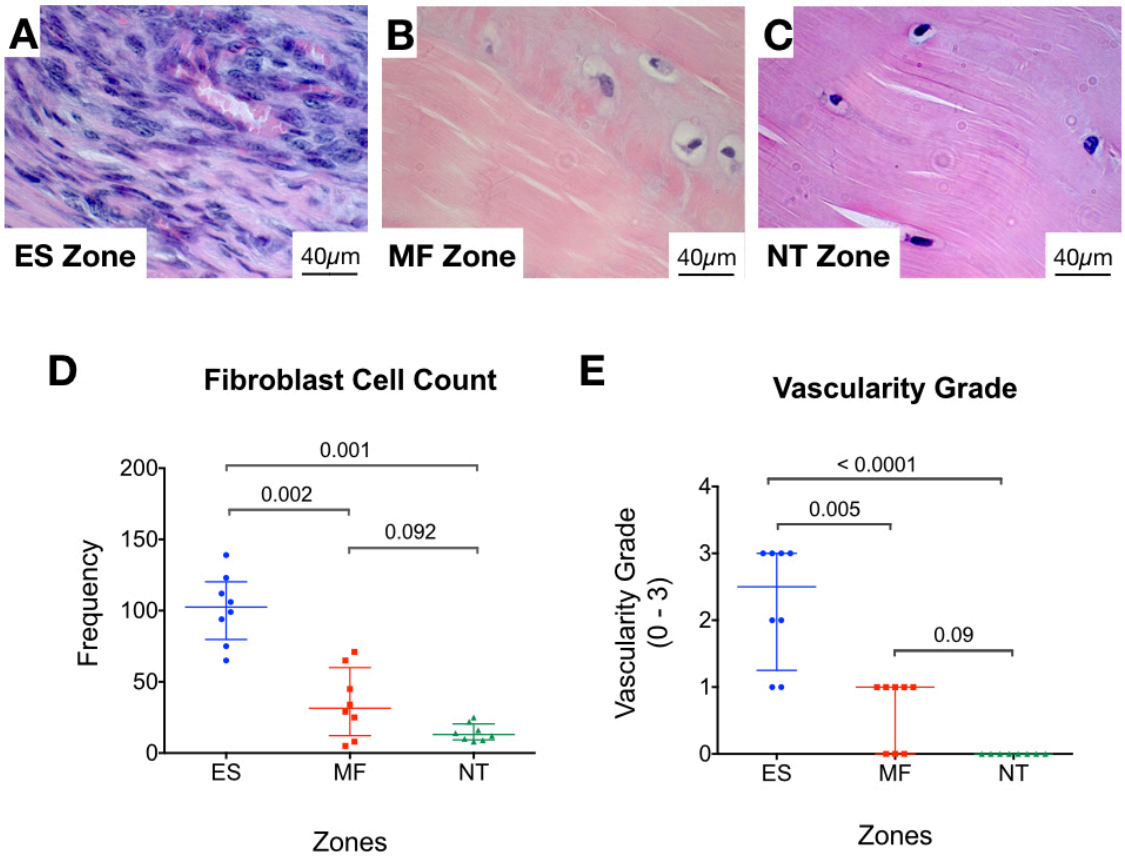


Figure 5-6: Cellularity and vascularity of electrospun suture implanted tendons. (A-C) Bright field microscopy images showing electrospun suture zone (ES) in A, tissue adjacent to monofilament suture zone (MF) in B, and normal tendon zone (NT) in C. Note high concentration of proliferative fibroblasts and several blood vessels seen in A. Also note similar appearance of scant well-orientated fibroblasts and no blood vessels in MF and NT zones. D) Graph showing median fibroblast cell count in ES, MF, and NT zones. E) Graph showing median vascularity grade in ES, MF, and NT zones. P values (Mann Whitney U test) shown above compared zones.

5.5.5 Adverse events

Two sheep demonstrated post-operative lameness and were euthanised at 1 month post-surgery in accordance with standard protocols. This was due to irritation from the distal suture knot (MF suture in both sheep) within the relative constricted area of the tendon sheath at the level of the metacarpophalangeal joint (fetlock canal). The ES suture was inserted proximal to the control suture in two further sheep to avoid similar problems. All other sheep were active and recovered uneventfully from surgery. Two other sheep, who were active and mobile following surgery, were noted to have a retained knot within a fibrous capsule (Figure 5-7 A/B). On histology, these retained knot areas, adjacent to the healing tendon, were also infiltrated with fibroblasts (Figure 5-7 D).

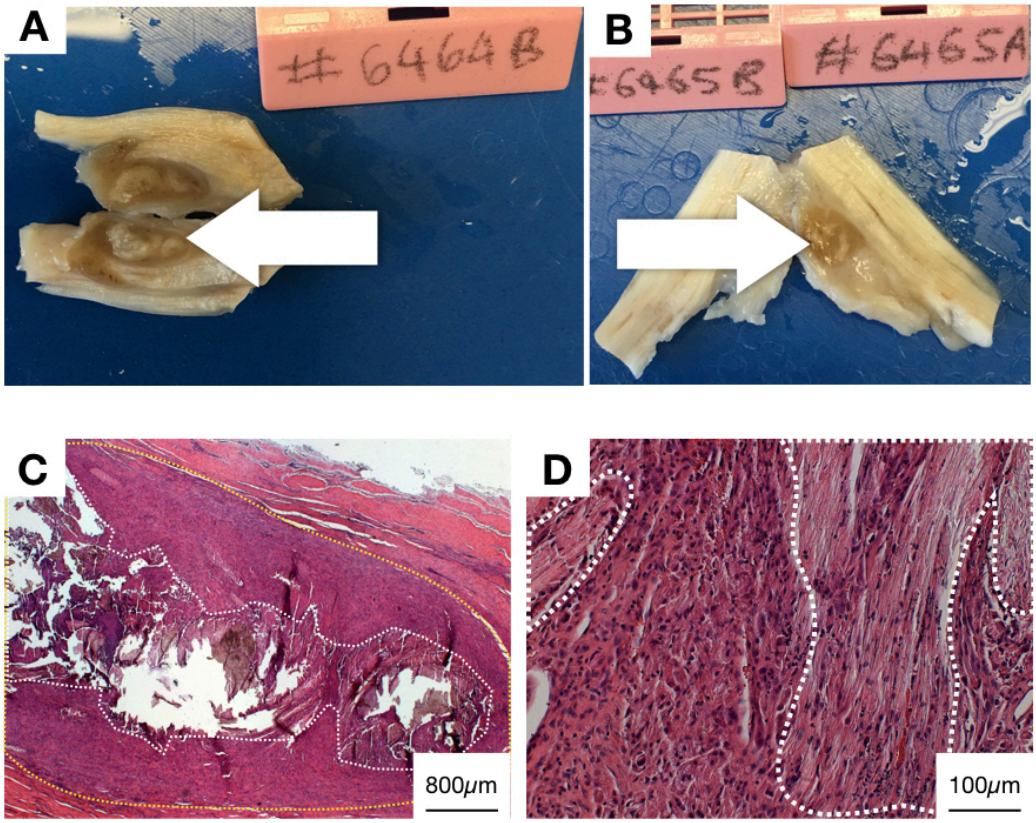


Figure 5-7 Harvested ovine tendons with retained knots. A/B) Digital photographs showing retained ES suture knot (white arrows) adjacent to DDFT tendon, encapsulated by fibrous capsule. Seen in two sheep, both were active and did not demonstrate any lameness postoperatively. C) Low magnification (2.5X) bright field microscopy image of H&E stained tendon harvest sample from sheep 6464 showing retained degrading polymer (dotted white boundary), surrounded by area of highly cellular tissue (dotted yellow boundary). D) Higher magnification (20X) bright field microscopy image of the same sample as in C, showing degrading polymer fibres (dotted white boundary). Fibroblasts seen here are more aligned than where polymer fibres are not visible (outside dotted white boundary).

5.6 Discussion

The multifilament electrospun suture used in repair of a tendon defect demonstrated both safety and efficacy in an ovine model of surgically-induced tendon injury. All tendon repairs healed on macroscopic inspection, with a mild local inflammatory reaction, minimal adhesions, and normal appearance synovial fluid. No adverse systemic response to suture implantation was seen on haematology, serology testing, or at necropsy. Compared to the MF control, the ES suture demonstrated significantly greater cellular infiltration and neovascularisation. Two adverse events were noted, due to the distal position of a large suture knot causing local irritation within the constricted region of the tendon sheath, even after transection of the palmar annular ligament. Two further sheep demonstrated a retained knot within a fibrous capsule around a healed repair. These sheep were active and mobile without any observed lameness.

5.6.1 Cellular infiltration into electrospun PDO sutures

Histological evaluation revealed significantly different tissue responses in the electrospun sutures compared to the monofilament sutures, 3 months after surgical repair. ES sutures were completely infiltrated with predominantly proliferative fibroblasts, and scant immune cells. In contrast, the MF control suture did not integrate into the host tissue and was occasionally seen in situ within the tendon on histology. On sectioning the harvested tendon tissue, the MF suture was frequently extracted from the section by the microtome, reflecting

a lack of integration with the surrounding tendon tissue. H&E staining also showed fragments of degrading PDO polymer within a tendon defect, without any cellular infiltration. Similar findings in a previous study utilising an infraspinatus repair model in Lewis rats were seen. Cellular infiltration into the electrospun suture from week 1 was observed whilst no infiltration in the monofilament suture (Prolene) was seen[191].

Other groups have shown similar cellular infiltration with a twisted electrospun polycarpolactone (PCL) yarn to repair flexor digitorum longus tendon defect in a murine model[262], and an electrospun Poly Lactic-co-Glycolic Acid (PLGA) scaffold in an infraspinatus repair model in rats[278]. In the current study, very few foreign body giant cells (FGBCs) were observed around the electrospun and monofilament sutures at 3 months. In a previous study in Lewis rats, FBGCs appeared by week 6, and resolved to negligible levels by week 12[191]. Using a different model, Kalfa et al also showed a peak of FBGCs, at 1 month, in response to an electrospun PDO patch, that resolved completely by 3 months[260]. In this study, given the single time point, it is not possible to determine whether FBGCs were present prior to the 3 month time point. It is reasonable that some foreign body giant cells are present to remove the polymer material as it degrades by hydrolysis over time. The fine filaments of the electrospun fibres were not always seen in the specimens, indicating that the vast majority of the polymer had degraded. Given the tendon defects have all healed at the 3 month time point used in this study, this rate of degradation is not of concern. However, this may not be the case in the human rotator cuff. Tears that fail to heal after

surgery can be identified by imaging within the first 6 month post-operative period[279]. Hence, to demonstrate clinical efficacy, these electrospun sutures would be required to induce healing in rotator cuff tears within their relatively short degradation time *in vivo*.

5.6.2 Neovascularisation of tissue integrated into electrospun PDO sutures

The ovine deep digital flexor tendon is similar to the normal human rotator cuff, in that it is generally hypovascular. A single capillary in the central core of the tendon is often seen in normal sheep. There are occasional small blood vessels in the paratenon surrounding the tendon. In this study, accompanying cellular infiltration, ES sutures supported a significant number of new blood vessels in the new tissue within the scaffold structure. Tissue adjacent to the monofilament control suture did not demonstrate a significant rise in blood vessels compared to the normal tendon zones. Therefore, the increased vascularity in ES zones is likely due to inherent bioactivity rather than a reaction to the injury induced by implantation of a foreign material.

Other studies have commented on neovascularisation in tendon repair after implantation of electrospun polymer tubes[280 281], or electrospun collagen scaffolds[258]. One study made a comment of vessel formation in the mid portion of an implanted electrospun PLGA scaffold in tendon repair, but this was not quantified[278]. To the contrary, several studies have not shown any neovascularisation within electrospun scaffolds used in tendon repair, even when additional growth factors are loaded onto the scaffolds[262 282]. Other studies

have applied electrospun scaffolds in different tissue types, including as vascular grafts, and demonstrated neovascularisation[267 283 284]. Other research groups have successfully stimulated neovascularisation by the addition of cytokines, growth factors, and stem cells to electrospun scaffolds[280 285 286]. At 3 months, the electrospun PDO suture, without the addition of growth factors or cytokines, demonstrated significant neovascularisation in the repaired ovine tendon. Hence, the neovascularised neotissue observed within our electrospun PDO suture is a key outcome parameter for effective tendon repair. In humans, blood vessels are more prevalent in small and medium tears compared to control (normal) rotator cuff tendons[41]. However, larger tears are hypovascular with a lower mean vessel count than the healthy tendon control[41]. Whether this neovascularisation in the sheep tendon will be replicated in the human rotator cuff tendon is yet to be determined.

5.6.3 Understanding adverse events of electrospun PDO sutures

Two sheep demonstrated lameness and were euthanised 1 month post-surgical repair. After modification of the technique to avoid the large MF suture knot irritating the constrained tendon sheath, the remaining sheep healed uneventfully. At 3 months post repair, we noted a widening of the tendon contour reflecting a fibrous capsule surrounding a retained knot in 2 sheep. The ES sutures used in this study were of large diameter (mean diameter 1.31mm), intended for tendon repair in humans. Our model involved repair of a longitudinal defect in the deep digital flexor tendon, which is confined in a sheath and surrounded by synovial fluid. Whilst this made for an ideal model to investigate

tendon repair as it is analogous to human disease, where defects fail to heal without adequate intervention, it also meant that the ES and MF sutures used may be too wide for this particular application in sheep. Histology of these two specimens showed that even the retained knot was heavily infiltrated with fibroblast cells and some foreign body giant cells.

5.6.4 Limitations

This study has several limitations due to the need to minimise the number of animals utilised. First, the response to the sutures was investigated at just one time point, 3 months post-surgery. Therefore, it is not possible to determine temporal changes of the healing tendon. However, a previous study conducted in rats, did show cellular infiltration of tendon fibroblasts from weeks 1 to 20 post repair, as well as a transient FBGC response that peaks at week 4 and resolves to negligible levels by week 12[191], which likely explains the lack of FBGCs in this study. Second, fibroblast numbers were determined based on histomorphometric features due to lack of specific and sensitive immunohistochemical cell markers. All of the commonly used cell markers for tenocytes and fibroblasts has been shown to be expressed in various immune cell populations and therefore are not reliable in identifying these cells exclusively. Third, while neovascularisation within the ES sutures was observed, conclusions as to whether this observation is positive or negative with respect to tendon healing cannot be made. However, vascularisation is considered important for repair, especially in tendon tissue. Massive tears have been shown to have the lowest healing rates in patients undergoing rotator cuff repair. Histologically, massive tears have significantly

less vascularity compared to other smaller tears and normal tendons[41]. One hypothesis for why massive tears fail to heal is related to this observation of significantly absent blood vessels. Therefore, in testing this hypothesis, the neovascularisation promoted by these ES sutures may play role in their efficacy to induce tendon healing. The mechanism by which neovascularisation occurred, however, remains unclear. Finally, the MF control suture was used as an internal control, which was placed adjacent to the ES suture in the same tendon defect where there is a risk of confounding effects from the adjacent suture material. A superior control would be to include sheep using only a control or ES suture to repair the defect. Whilst this may influence macroscopic observations of the repairs, the differences in histology reflect more localised cellular responses to suture materials that is less likely to be due to the effects of adjacent sutures.

5.7 Conclusions

In a large animal non-healing tendon defect model, electrospun PDO sutures promoted effective tendon healing, fibroblast cell infiltration, and neovascularisation compared to conventional monofilament PDO suture (control). No adverse systemic features were noted at 3 months post implantation. On inspection, all lesions healed with a mild local inflammatory reaction, minimal adhesion to surrounding tendon sheath, and normal-appearance synovial fluid. Careful consideration of the placement and size of sutures used in this particular model should be made to avoid adverse events seen with knot retention and MCPJ irritation. A first-in-human clinical trial

investigating the safety and efficacy in tendon repair is the next translational step toward future patient benefit.

5.8 Future work

Future work will focus on testing hypotheses generated from the findings of this chapter. Whilst the ovine tendon repair model used in this study fails to heal without surgical intervention, representative of human rotator cuff disease, it is still important to test how these sutures affect the reconstruction of the tendon-bone enthesis. No animal model adequately replicates the complexity of function of the human shoulder, however, understanding how these bioactive sutures behave at the tendon-bone interface would add evidence to their application in human rotator cuff repair.

Further questions also remain to be investigated. This chapter focussed on the tissue response to electrospun PDO sutures, however, the effect of cellular infiltration into these sutures on the biomechanical properties of the healing tendon is unknown. It is not known whether the infiltration of tissue seen in this study will lead to less cheese-wiring of the suture through the tendon. Furthermore, it is unknown if a healed tendon repaired with an electrospun suture is ultimately stronger than one repaired with a conventional monofilament suture.

The evidence presented in this chapter is a component of what is required to move towards careful application in a first-in-man trial. The electrospun PDO

suture is currently undergoing further biological evaluation, including testing for genotoxicity, *in vitro* cytotoxicity, sterilisation residuals, and quantification of degradation products, as outlined by the BS ISO 10993-1:2009 guideline "Biological evaluation of medical devices". In addition, a first-in-man trial may take several forms and requires approval from the Medicines and Healthcare products Regulatory Agency (MHRA). A small number of carefully selected patients, informed of the experimental nature of these sutures, would be recruited. Similar to the *in vivo* study in this chapter, systemic, local, and cellular response should be assessed at an appropriate time point. The focus will be to demonstrate that these sutures are safe, non-toxic, and capable of being utilised in rotator cuff repair surgery in humans. Given these sutures are made from Polydioxanone, a polymer that has been employed in conventional sutures used in surgery since 1982[276], it is not expected that the electrospinning process will alter their safety profile.

Following this safety trial, a clinical efficacy study may follow. This randomised controlled trial would be designed to investigate whether these electrospun sutures improved healing rate at a defined time point, e.g. 6 months post-surgery, compared to a control group receiving standard repair. As with most clinical efficacy trials, strict inclusion and exclusion criteria would be defined and adhered to. The aim is to minimise as many controllable factors that may have even a small influence on the healing rate. If these electrospun PDO multifilament sutures demonstrate clinical efficacy in optimal circumstances, a second, larger clinical and cost effectiveness randomised controlled trial would

be required to demonstrate external validity when the general cohort of shoulder surgeons employ these sutures in a variety of rotator cuff repair constructs. Here the primary outcome may be Oxford Shoulder Score (OSS) at 1 year for example, with healing rate being a secondary outcome.

5.9 Clinical implications

The clinical implications of this novel suture begin with its initial design brief, improving the likelihood of healing following rotator cuff repair. Incorporating this suture into suture anchors currently in clinical use, will be determined by the calibre of the final suture for implantation. Most suture anchors will accommodate between 2-4 sutures depending on size[287-290]. Most repair techniques can still be performed in a similar manner utilising these bioactive sutures rather than the preloaded synthetic inert sutures currently being used. The wider implications of a bioactive suture that becomes incorporated with host tissue are numerous. The advantages of tissue integration, neovascularisation, and full degradation over time, can be applied to multiple tissue types and a wide range of surgical procedures. Another question that arises, given that this chapter and the preceding one demonstrated the bioactivity of both an electrospun patch and electrospun suture for tendon repair, is which to use and when?

5.9.1 Electrospun sutures vs electrospun patches for tendon augmentation

The application of either electrospun sutures or electrospun patches for rotator cuff repair augmentation have advantages and disadvantages. These are summarised in Table 5-3.

Table 5-3: Comparison of advantages of utilising electrospun PDO sutures and electrospun PDO/PCL patches in rotator cuff repair surgery.

Advantages of applying electrospun PDO sutures	Advantages of applying electrospun PDO/PCL patches
Can be easily incorporated into current rotator cuff repair surgery without alteration in technique	Larger volume for tissue integration and neovascularisation
Complete suture will degrade at similar rate throughout	Additional structural support for healing tendon
Closely applied to tendon-bone interface without any gapping	Protective effect of interposition between healing tendon and bony acromion above
No additional time required to complete repair	

If both electrospun devices are licensed for use, a clinical efficacy trial comparing them would be useful to inform clinical application. For example, a

four-arm parallel group randomised controlled trial may determine the impact of healing of these implants in rotator cuff surgery.

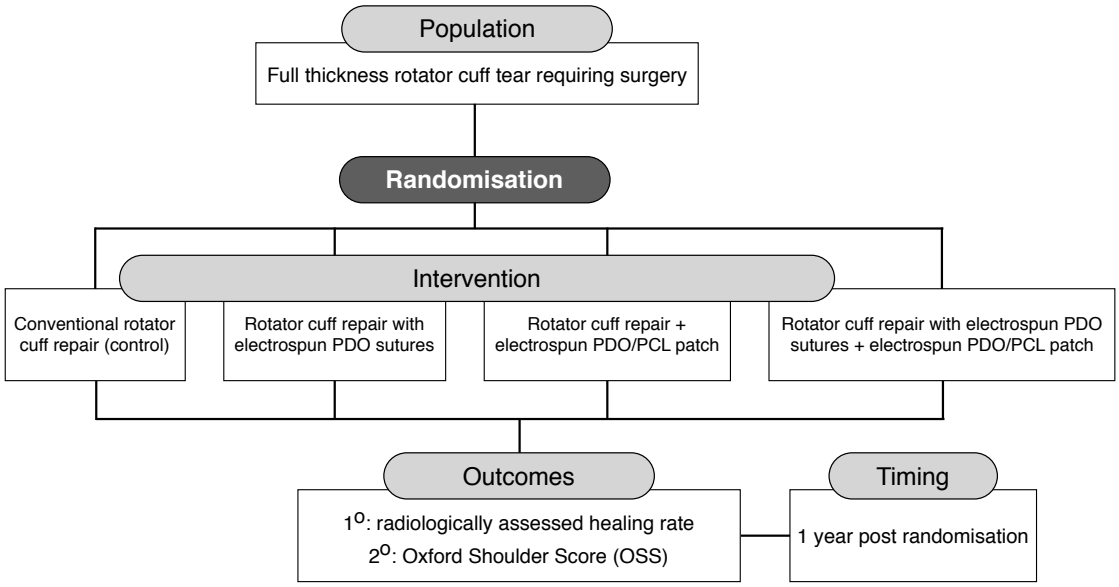


Figure 5-8: Example of possible trial design for assessing clinical efficacy between electrospun implants used in rotator cuff repair.

This concludes the final experimental chapter. The next chapter will provide a general discussion and summarise the key findings of this thesis.

Chapter 6: General discussion

This final chapter will discuss the experimental findings presented in chapters 2-5. Following this, the implications of this work and future work will be outlined. The limitations of this thesis will be discussed. To conclude, a summary of the key findings will be presented.

6.1 Rotator cuff healing

Asymptomatic tears of the rotator cuff tendon are common[22]. Occasionally, a traumatic event may be associated with a painful rotator cuff tear however, given the high rate of asymptomatic cuff tears in over 60 year olds, it is unclear whether this traumatic event caused the tear or converted a previously asymptomatic tear to a symptomatic one. A significant proportion of cuff tears occur without any preceding traumatic event[202]. Hence, trauma cannot solely explain the aetiology of these injuries. Histology of cuff tears has shown significant changes from a normal healthy tendon[41]. Torn tendons have often experienced recurrent episodes of inflammation and subsequent fibrosis that likely preceded the structural defect that surgeons identify clinically, radiologically, and at the time of surgery. It is clear from torn tendon tissue samples that not all tear sizes are the same histologically[41]. Massive and large tears contain fewer fibroblasts, fewer blood vessels, and the presence of chondroid metaplasia[41]. Clinicians have also identified the sequelae of rotator cuff tears, more often seen in larger tear sizes[219]. These are thought to be related to unloading of the tendon-muscle unit. The observations of muscle atrophy and fatty infiltration of the rotator cuff muscles has been correlated with

poorer functional outcomes and lower healing rates, however no study has demonstrated the mechanism by which these findings occur[124 205 216 218].

Rotator cuff healing can be defined in several ways as outlined in chapter 1. The gold standard of repeat arthroscopy for the purposes of determining healing is not feasible. Various radiological methods of assessment have been proposed as alternatives, however, they are often considered inaccurate. An additional consideration is when to assess healing following surgery. Iannotti et al demonstrated by serial ultrasound scanning that the majority of cuff repairs fail in the first 6 months after surgery[279]. There is also a discrepancy between the observed reduction in pain following rotator cuff repair surgery and radiologically assessed healing[202]. Patients will often report a significant reduction in pain following cuff repair, even if the repair is found to have failed to heal radiologically[202]. In addition, 85% of patients will report improvement in symptoms 2 years after surgery, despite 60% of these repairs remaining attached to the footprint at 1 year[202]. This has led some surgeons to question whether healing is important for pain relief and function[122 291]. These conclusions are drawn from observational studies that often include unmitigated biases, such as attrition and detection bias, leading to sometimes inaccurate conclusions. The UKUFF trial, a large well-conducted randomised controlled trial, demonstrated a clinically significant increase in patient reported outcomes measures in patients with a healed repair compared to those with a repair that failed to heal[202]. Therefore, healing following rotator cuff repair is a meaningful goal for clinicians attempting to achieve maximal benefit for their patients.

6.1.1 Determining the healing rate following rotator cuff repair

Before considering methods in which to improve the healing rate, we must first define what the healing rate is. Surgery is a complex intervention, one that is influenced by a plethora of factors[216]. Additionally, rotator cuff repair is a challenging surgical intervention that requires significant skill and refinement to achieve reproducible success. The significant bias in study design in observational studies reporting healing rates has yielded a range between 6 and 94%[103 104]. There are some randomised controlled trials that have reported healing as an outcome[32 110 111 136 138 139 144]. Most of these studies have a moderate-to-high risk of bias. However, the most recent, the UKUFF trial, involved 65 surgeons over 47 hospitals in the UK. This study reported an overall healing rate of 60%[24]. This figure for the healing rate is likely a true reflection given the large number of surgeons and hospitals that were involved in the study.

6.1.2 Factors influencing healing rate

Numerous studies have reported a correlation between certain factors and healing rate. The vast majority of these studies are observational, with inherent methodological biases. For most factors, there are studies supporting and refuting their influence on rotator cuff repair healing. The commonest cited are age, tear size, and muscle atrophy/fatty infiltration. Several randomised controlled trials have reported differences in mean age of patients with a healed repair compared to those without a healed repair[32 107]. However, these trials do

not comment on the relationship between age and tear size. Tear size has long been considered an important factor influencing healing by surgeons[104]. Anatomic repair is often much harder to repair for larger tears, due to tendon edge retraction, immobility of the tendon, and poor-quality tissue prone to cheese wiring[292 293]. Additionally, histologically, larger tears have a reduced capacity for healing[41].

Given the degree of dissonance in the published scientific literature, the influence of age and tear size on healing was investigated in the first experimental chapter of this thesis (Chapter 2). Using data from the UKUFF trial, it was demonstrated that age was an independent factor influencing healing. For patients undergoing rotator cuff repair surgery, increasing age correlated to decreased likelihood of rotator cuff repair healing. Using a logistic regression model, it was demonstrated that due to the relationship between age and tear size, only massive tears were independently predictive of reduced healing rate. Additionally, a predictive model for healing at 1 year for an average 50, 60, 70, and 80-year-old patient with a small, medium, large, or massive rotator cuff tear was generated. This predictive model showed that not only older patients with larger tears would benefit from repair augmentation to increase the likelihood of healing, but that even small tears in younger patients may benefit. This chapter provides some useful insights to clinicians treating patients with a rotator cuff tear. Principally, in consulting with patients about the outcomes of surgery, surgeons are now able to provide a better reflection of the estimated probability of healing at 1 year. Additionally, this research will add to the evidence that not

all tears heal, which is an important consideration in rotator cuff tears. Finally, if we appreciate that not all tears heal, we may then consider how best to augment rotator cuff tendon repairs in order to improve the healing rate. More importantly, an appreciation that a solution is required, with regards to rotator cuff repair healing, means further research in this area may be conducted to fulfil this unmet clinical need.

6.1.3 Response of native supraspinatus tendon to biological patches for augmentation

Several strategies have been proposed to improve the healing rate following rotator cuff repair. Patch augmentation whereby a graft material is laid on top of a repaired tendon to improve healing is a popular augmentation strategy. Biological patches, made from decellularised dermal tissue, either allograft or xenograft, have been used by surgeons for rotator cuff repair[294 295]. In the second experimental chapter (Chapter 3), an investigation of the native tendon tissue response to two commercially available biological patches in clinical use was performed. At 4 weeks after implantation, significant extracellular matrix (ECM) disruption was observed in the supraspinatus tendons augmented with the dermal allograft or dermal xenograft patches. The ECM disruption caused by the dermal xenograft patch was more significant than that seen by the dermal allograft patch. These biological patches tested did not increase the vascularity, cellularity, or foreign body giant cell count at 4 weeks, although the sample sizes in each group were small. One patient that received a dermal xenograft patch had an abnormal tissue response accompanying a clinical picture of painful, stiff,

shoulder following implantation that resolved spontaneously with analgesia and anti-inflammatory medication. This patient's tendon tissue, in response to the patch, showed dense infiltration of pro-inflammatory immune cells, particularly $CD68^+$ / $CD206^+$ / $IRF5^+$ cells, suggestive that these are tissue resident macrophages with a pro-inflammatory phenotype. This is the first study to assess the early tissue response after biological patch augmentation in humans. In addition to the concerning findings presented in Chapter 3, high quality clinical efficacy studies demonstrating superior clinical outcomes of these biological patches are lacking.

Biological patches are derived from different cadaveric and animal sources. They are decellularised and processed by proprietary processes that are not well defined. Hence, there is a natural variation between patches that may influence the tissue response. Additionally, these biological grafts are often dermal or small intestine submucosa in nature, and are not specifically designed for tendon augmentation. Synthetic patches used clinically for rotator cuff repair augmentation have focussed on providing structural support to the healing tendon[160]. They are usually manufactured from non-biodegradable polymers, thought to be inert. In reality, all synthetic polymeric implanted materials interact with the recipient's immune system, leading a cascade of events that leads to maturation of granulation tissue and fibrous capsule formation[247].

6.1.4 Response of tendon injury to electrospun synthetic patches for augmentation

Biodegradable polymeric scaffolds, such as the one investigated in the third experimental chapter (Chapter 4) demonstrated bioactivity. In a non-healing ovine model, a novel electrospun PDO/PCL patch was tested for safety and efficacy. This biodegradable synthetic patch induced tendon repair in this model, without a systemic or adverse local response. The electrospun fibres were well integrated with predominantly proliferative fibroblast cells and rarely, foreign body giant cells. Some immune $CD45^+$ / $CD14^+$ / $TGF\beta1^+$ cells were observed. Significant neovascularisation was a key finding of this study, which showed this collagen-containing ECM tissue was well vascularised. Whilst the mechanisms for these observations need to be identified, the results are promising for an alternative synthetic patch augment that has been designed for tendon repair using strategic biomimicry.

A biodegradable synthetic patch has the advantages of potentially avoiding the fibrous capsule formation seen following chronic inflammation in non-biodegradable patches. In addition, synthetic patches have no biological components and therefore can be manufactured to meet strict guidelines, reducing the variability seen in biological patches. Neovascularisation may be a key feature for tendon augmentation, especially in larger tear sizes that have been shown to have fewer blood vessels than normal tendons[41]. The electrospun PDO/PCL patch investigated promoted neovascularisation in the tissue integrated within the scaffold, 3 months after repair. Contrary to biological

patches, some of which have demonstrated a strong foreign body immune response, this synthetic patch did not show a systemic response or adverse local response. Foreign body giant cells were rarely seen at 3 months. The clinical implications and future work proposed for this implant will be discussed later in this chapter.

6.1.5 Response of tendon injury to electrospun synthetic sutures for repair

Having demonstrated a favourable tissue response to an electrospun PDO/PCL patch, an alternative strategy, using the same electrospinning technology applied to suture manufacture was employed. This electrospun multifilament PDO suture was tested in a similar ovine model as that in Chapter 4. The findings of cellular infiltration and neovascularisation in the electrospun fibres of the patch were also seen in the electrospun (ES) PDO suture. Compared to a PDO monofilament (MF) control suture, this novel suture demonstrated a significantly different tissue response. Principally, tendon fibroblasts infiltrated into the ES suture whilst they did not in the MF suture. This infiltration was accompanied by significant neovascularisation which was not seen around the MF suture. These findings demonstrate that the electrospun PDO suture was bioactive and promoted a favourable cellular response compared to the relatively inert PDO monofilament suture. In addition, no systemic or adverse local response was noted. This investigation however did illustrate adverse events related to knot size and location. In retrospect, the monofilament sutures were implanted too proximally in 2 early sheep that demonstrated lameness from knot irritation of the surrounding sheath. Additionally, 2 further sheep retained knots

at 3 months. This was likely related to suture diameter being too large for this particular application as it was intended for human rotator cuff repair, which has relatively more space than the narrow tendon sheath of the deep digital flexor tendon in sheep. Despite these retained knots, these sheep did not demonstrate significant lameness and tissue sections at 3 months showed these knots were infiltrated with fibroblasts.

The findings of the electrospun PDO/PCL patches in Chapter 4 and the electrospun PDO sutures in Chapter 5 are encouraging. These studies were conducted in a non-healing, intra-synovial, large animal model of tendon injury. Further biological evaluation is required to meet the necessary standards for licensing for implantation in humans. Safety and clinical efficacy in rotator cuff repair augmentation in humans is required in both implants before widespread use is recommended. Many prior augmentation devices have been tested to meet the necessary requirements for licensing regulations but have little, if any, clinical efficacy evidence to support their use.

6.2 Implications of this work

The findings presented in the experimental chapters of this thesis have implications for the treatment of rotator cuff tears. The clinical and academic challenges presented by rotator cuff tears are still present. Research presented in this thesis may be utilised by clinicians and researchers alike. Clearly, further research is required to identify strategies that improve healing following rotator

cuff repair. In this section I will outline how the work presented in this thesis may impact patients, surgeons, and researchers.

6.2.1 Patients with a rotator cuff tear

Patients with a rotator cuff tear are not a homogenous group. Expectations of treatment often varies from reduction in pain to acceptable levels, to return to recreational sport or manual work. Not all patients with a cuff tear will present to see a medical professional, as many are asymptomatic[22]. Symptomatic patients failing conservative treatment may be counselled about the risks and benefits of surgical intervention. The findings in Chapter 2 may be used to help provide patients with more tailored information about the rate of healing based on their age and tear size. However, there are several factors to consider here. First, although a healed repair provides a superior clinical outcome to a non-healed repair, the vast majority of patients will report a clinically significant benefit from surgical intervention[202]. This is likely due to other factors such as the time taken off work/activity following surgery, the rehabilitation regime, physiotherapy input that patients' experience following surgery, and the placebo component of the surgical intervention. Most patients undergoing surgery in the UK will have symptoms for many months (mean 2 - 2.7 years), but regression to the mean with regards to pain is still a factor[202]. Second, the predictive model described in chapter 2 is a guide only, and still requires validation in a separate population of patients. Hence its use should be taken with some degree of caution. Third, the tear size identified radiologically prior to consideration of surgery, using radiological assessment, is not as accurate as the assessment of tear size used

in Chapter 2 (surgeon assessment intra-operatively)[36 296]. Therefore, using the predictive model to inform patients of their likelihood of having a healed repair at 1 year based on their age and tear size will be affected by this inaccuracy. Fourth, the predictive model described in Chapter 2 is useful to provide an estimate of healing however we cannot definitively identify which patients' repairs will heal and which will not. Finally, patients play an important role in designing, conducting, and disseminating high quality research. As such, any clinical trials should seek to recruit patients willing to participate and contribute to the trial in all areas of design and conduct[297].

6.2.2 Surgeons treating rotator cuff tears

For surgeons treating rotator cuff tears, there are important implications of the evidence presented in this thesis. Firstly, Chapter 2 illustrates that not all rotator cuff tears heal following surgery. This is an important finding and not one that is universally held by all surgeons. As the vast majority of patients report clinical improvements following surgery, and routine care does not include a radiological assessment of repair healing, surgeons may be misled to believe that a much higher proportion of patients' repairs remain intact at 1 year. The healing rate as mentioned in the previous section is influenced by age and tear size. The predictive model can be used carefully by surgeons to not only inform consultations with patients but also to reconsider their decision-making processes for surgical intervention. Perhaps fewer conventional unaugmented repairs will be considered in older patients given the low rates of healing. Surgeons are not only technicians, but rather scholars and collaborators[298].

Hence, engagement with developing and investigating augmentation strategies is important in order to advance our understanding of whether these strategies affect tendon healing.

The findings in Chapter 3 should be taken in context of the low numbers in each group however, the negative impact on extracellular matrix of the native tendon in response to the overlying dermal allograft and xenograft patches was consistent and concerning. This study demonstrated that it is feasible to harvest tendon tissue biopsy samples soon after surgical repair to gain insights into early tissue response. Future work relating to these biological augmentation patches will be described later in this chapter, but any clinical trial design will require involvement from surgeons willing to consider enrolment of their patients. The findings in Chapter 3 should serve as an important point for surgeons to consider further scientific validation before continuing clinical use of these patches. The patches investigated in this chapter are currently used by surgeons to augment rotator cuff healing, however they were initially developed to cover skin defects in foot ulcers and augment hernia repairs[156 157]. This chapter highlights the need for scaffolds designed to augment tendon healing, specifically the rotator cuff enthesis.

6.2.3 Future use of biological patches to augment rotator cuff repair

The investigation conducted and reported in Chapter 3 is the first of its kind to systematically evaluate the native tendon tissue response to two commercially available biological patches. These patches did not demonstrate increased

cellularity or vascularity in the native supraspinatus tendon. Similarly, a foreign body giant cell response was not observed. Immunohistochemistry for inflammatory, fibrotic, and immune cell markers did not show any differences between these patches and the control (no patch) group. However, the extracellular matrix disruption observed in the two patch augmentation groups was significant compared to the control group. As such, it was concluded that the overall tissue response to these patches was not favourable. Given the lack of high quality clinical trials demonstrating their efficacy, future implantations of these patches should ideally occur in the context of a well-designed randomised controlled trial. If their clinical efficacy on healing rate or patient reported outcome measures are demonstrated in such a trial, then their widespread application may be considered. A clinical trial may incorporate a tissue biopsy similar to that conducted in Chapter 3, at a later time point, to allow investigation of the tissue response to determine how these patches affect the native repaired tendon.

6.2.4 Development of electrospun synthetic scaffolds to augment rotator cuff repair

Chapter 4 and 5 are experimental and investigated two novel electrospun polymeric scaffolds. These are not licensed for use currently, and their clinical application may be some years away. However, the results from previously published studies[190] [191], and the evidence presented in this thesis, are encouraging. In ovine tendons, these implants were applied without any safety concerns. In addition, the healing tendon fully integrates into these electrospun

scaffolds, leading to significant proliferative fibroblast infiltration and neovascularisation. Translation to clinical use is foreseeable once further appropriate biological evaluation and regulatory approval is sought. Patch augmentation is currently performed infrequently in rotator cuff repair cases. Surgeons will undoubtedly experience a learning curve with the application of electrospun scaffolds for cuff repair augmentation. Therefore, careful clinical implementation is required and surgeons should play a role in the shift towards consideration of augmentation with these implants in the future.

6.3 Future work

There are several research questions raised by the findings presented in this thesis. In the first experimental chapter, the influence of age and tear size was investigated, leading to the creation of a predictive model for healing. As with any predictive model, this must now be validated using a new population of patients undergoing rotator cuff repair surgery. Age and tear size are factors commonly cited as influencing healing following cuff repair surgery, however, there are others. Muscle atrophy or fatty infiltration are another commonly cited prognostic indicator for healing. Assessment of supraspinatus muscle atrophy and fatty infiltration can be made in several ways[219 299-301]. In the UKUFF study, a systematic assessment of supraspinatus muscle atrophy or fatty infiltration was not made and recorded, hence data was not available to investigate this factor and its influence on the healing rate. However, it would be difficult to adjust for the confounder that some surgeons may not offer surgery in patients with severe muscle atrophy or fatty infiltration.

The second experimental chapter provides a useful precedent for a clinical study whereby patients undergo a biopsy of their repair soon after rotator cuff repair surgery. Due to small numbers, any significant difference between the groups could not be identified on histology and immunohistochemistry analysis. However, a deleterious effect on the extracellular matrix structure was identified in the biological patch augmentation groups. Given these implants are commercially available and are being used clinically by surgeons, it is appropriate to conduct a trial to investigate their clinical efficacy. The most robust way in which to investigate the clinical efficacy of a surgical intervention is a randomised controlled trial. This study design permits minimisation of potential sources of bias, and if conducted appropriately, will provide great insight into whether these biological patches improve healing rate and/or clinical outcomes.

The benefits of a synthetic degradable polymeric patch for rotator cuff augmentation have been highlighted. The novel electrospun PDO/PCL patch investigated in Chapter 4 showed, in a validated large animal model of tendon repair, that fibroblasts infiltrated the electrospun fibre layers. Additionally, the tissue integrated into the patch was accompanied by new blood vessels. These findings demonstrated the bioactivity of this scaffold, however there are several unanswered questions, which will be the focus of future work. First, the biomechanical properties of the electrospun patch augmented tendons are unknown. A biomechanical study would ideally investigate the influence of the patch on the healing tendon's load to failure and stiffness over time, as the

tendon tissue integrates with the biodegradable scaffold. Second, the neovascularisation phenomenon observed requires further research to better understand this phenomenon. Specifically, to determine the mechanism by which this neovascularisation occurred and whether this observation was advantageous to the healing tendon. *In vitro* studies using endothelial cells mounted on electrospun scaffolds would provide some insight as to the cell response and genes upregulated when these cells interact with this scaffold. *In vivo* experiments in animal models, not necessarily in tendon repair, will help validate the *in vitro* experiments and add an extra layer of understanding to the processes observed. For example, subdermal implantation of the electrospun PDO/PCL patch in a murine model would provide evidence of degradation properties *in vivo*.

Synthetic multifilament sutures are used in rotator cuff repair today. The findings reported in Chapter 5 confirmed that electrospinning of PDO polymer to form multifilament yarn converted an inert polymer into a bioactive suture, capable of cellular infiltration and neovascularisation. The electrospun patch and suture investigated in this thesis are undergoing necessary testing in preparation for regulatory body approval. This work is being undertaken by others in the research group and is not discussed in this thesis. Following regulatory approval, further research to demonstrate safety and clinical efficacy for use in rotator cuff repair in humans is planned. Following these studies, a clinical and cost effectiveness trial may be performed.

6.4 Limitations of this thesis

All research has limitations and compromises. Findings reported in this thesis must therefore be interpreted in the context of the limitations outlined in each experimental chapter. These limitations and the implications they have on the findings presented are discussed here.

6.4.1 Defining rotator cuff healing

Chapter 2 focussed on investigating the influence of age and tear size on rotator cuff repair healing. Assessing healing following surgical reattachment of the torn rotator cuff is difficult. Second look arthroscopy for the purposes of determining healing is unethical and unnecessary. The best alternative is to utilise a number of radiological imaging techniques. Magnetic resonance imaging (MRI) and ultrasound scanning (USS) are the two most popular methods used in the United Kingdom for assessing rotator cuff healing. There are other methods used in other healthcare systems, such as computed tomography arthrogram (CTA). Each method has advantages and disadvantages. The interpretation of MRI and USS scans in the context of the surgically-repaired shoulder is complex. There is a classification system often used, that categorises the status of the repaired tendon[91]. The intra-observer reliability has been determined as good (κ 0.64), but the inter-observer reliability is only fair (κ 0.34)[92]. USS imaging reliability is technician-dependent and will often require musculoskeletal expertise to provide sufficient accuracy in the context of assessing rotator cuff repair integrity[302].

The UKUFF trial was set up to maximise the accuracy of imaging assessment of the repairs, as much as possible. A single senior consultant musculoskeletal radiologist reviewed all scans centrally. Rather than using the Sugaya classification, repairs were determined to be intact, torn, or inconclusive. This simpler classification was thought to increase accuracy; however one limitation is that the accuracy of this assessment was not determined as part of the UKUFF trial.

6.4.2 Recruitment for biological patch augmentation study

The second experimental chapter (Chapter 3) investigated the tissue response of the native supraspinatus tendon that was augmented with either a dermal allograft patch (GraftJacket), a dermal xenograft patch (Permacol), or no patch (control). Recruitment into this study was challenging for several reasons. Patients are often very well informed about the options for surgery prior to discussion with their surgeon. Patch augmentation is not commonly performed and an additional step to a conventional repair that patients may have heard or read about previously. Hence, patients required time to have this study explained to them, and some were not willing to participate due to the requirement of a needle biopsy 4 weeks after surgery. These factors led to small numbers of patients being recruited into each group, some of whom did not provide tissue samples for reasons outlined in Chapter 3. This lack of power precluded statistical testing of the tissue analysis, hence this was not performed. Despite this, this was the first study to systematically collect tissue biopsies from rotator cuff repair patients soon after surgery. Therefore, this study

demonstrated that it is feasible to conduct research using tissue biopsies taken in the early phases of the post-operative recovery period.

6.4.3 Tissue biopsy following cuff repair

Performing tissue biopsies of the rotator cuff repair site is challenging and required the expertise of an experienced consultant surgeon that has performed similar biopsies for research purposes for many years. Despite this, and with adequate prior validation of the technique, there remains some limitations with the tissue biopsies harvested. First, the volume of tissue is limited and is determined by the gauge of the core biopsy needle. Therefore, a relatively small number of sections are cut from these postoperative samples compared to the intra-operative samples that are harvested during surgery. This limited the amount of staining, both in histology and immunohistochemistry, that could be performed. Second, the biopsy needle passes across the repair site, through the patch (if applied), and into the native underlying supraspinatus tendon. The biopsy samples consistently harvested the native supraspinatus tendon, whilst only some showed both the native supraspinatus tendon and the patch tissue. Hence, the tissue analysis in Chapter 3 focussed on only the native supraspinatus tendon tissue response as this was identified in all post-operative samples.

6.4.4 Tenocyte specific markers

Immunohistochemistry on human rotator cuff tissue and sheep digital flexor tendon samples was performed in the experiments reported in this thesis. In both situations, there is a distinct lack of sensitive and specific tenocyte or tendon fibroblast markers available for use in immunohistochemistry[303]. In Chapter 3, total cellularity was used as a surrogate measure for fibroblast cell count. In Chapters 4 and 5, a reliance on histomorphometric features was required to identify fibroblast cells and immune cells, however there were significantly fewer immune cells recorded. Most commonly cited tendon fibroblast IHC markers are also variably seen in other cell types, specifically immune cells[303].

6.4.5 Animal models for rotator cuff healing

Chapters 4 and 5 utilised an ovine model developed at the Royal Veterinary College (RVC). This model has been validated, demonstrating that the surgical defect created in the tendon failed to heal without adequate intervention at 6 months[253]. This finding is rarely seen in other animal models, especially rodent models. In murine models, the infraspinatus tendon is the predominant shoulder tendon, and will often regenerate after excision over time. Hence, most experiments performed using these models will use a short time point (4 weeks or less) to identify a difference between groups before the infraspinatus tendon has healed spontaneously[304]. The ovine model is a compromise however. Firstly, this model does not involve the enthesis, which would have been

desirable as these implants were designed with human rotator cuff repair in mind. Secondly, sheep are quadrupeds and load their limbs differently to humans. The ovine deep digital flexor tendon is an energy-storing tendon whilst the rotator cuff is a positional tendon, hence differences in tendon type and function exist. Despite these limitations, this model did serve to allow tendon repair to occur in a synovial compartment, and allowed demonstration of healing that does not occur spontaneously.

6.4.6 Control patch in tendon repair

In Chapter 4, an electrospun PDO/PCL patch was implanted overlying an ovine tendon defect. In that study, an internal control was used. An area of tendon tissue distant to the repair but in the same tendon, was imaged. This was referred to a NT or “normal tendon” zone. A superior control would have been to create a defect that is then left untreated, however this would have significantly increased the number of animals required to complete the study and therefore not compliant with the principles of replacement, reduction, and refinement in animal research (The 3 Rs)[305]. Additionally, the electrospun fibre zone demonstrated significant fibroblast cell infiltration and neovascularisation. To conclusively demonstrate that the electrospun fibre layers of the implant produced this response *in vivo*, a similar implant with the woven PDO layer only, could be implanted to serve as a control. This would be akin to the PDO monofilament control used in Chapter 5, however due to the size of the tendon defect, it would require placement on a different tendon, which means a doubling of the number of animals required.

6.5 Key findings of this thesis

Four experimental chapters presented here range from clinical studies to animal *in vivo* experiments. The first experimental chapter investigated the influence of patient age and tear size on healing following rotator cuff repair, using data from a large clinical trial. The key findings were that age and tear size both influenced healing rate at 1 year post-surgery; age was the predominant factor. More importantly, the predicted healing model generated in this chapter presents a powerful argument for the need for rotator cuff repair augmentation. These findings are represented graphically in Figure 6-1 below.

The second experimental chapter focussed on investigating the tissue response of the native supraspinatus tendon 4 weeks after repair and augmentation with one of two biological patches. This study demonstrated significant extracellular matrix disruption in both biological patch augmentation groups compared to the control (no patch) group. Additionally, these patches did not increase cellularity or vascularity in the native tendon.

The third experimental chapter investigated a novel electrospun biodegradable polymeric patch for tendon repair in an ovine non-healing model. This electrospun biodegradable patch demonstrated bioactivity by inducing repair, fibroblast cell infiltration, and neovascularisation. This implant was also shown to be safe as it did not produce a systemic response at 3 months, and the

macroscopic inspections were in keeping with a mild local inflammatory response.

In a similar ovine model, an electrospun biodegradable polymeric suture was tested against a monofilament control suture made of the same material. At 3 months, these sutures demonstrated significantly different tissue responses. The electrospun suture promoted cellular infiltration, predominantly of fibroblast cells, and neovascularisation. The monofilament suture was extracted from the tendon sample during sectioning due to lack of integration. When seen *in situ*, there was no cellular infiltration or neovascularisation observed in relation to the monofilament control suture.

This thesis focuses on the clinical and academic challenges of rotator cuff tendon healing and augmentation. Some useful insights have been garnered from the experiments conducted in Chapters 2-5. However, more research is required in order to achieve a safe, sustainable, and efficacious solution for patients with a rotator cuff tear. The goal remains to achieve a healed rotator cuff tendon in all patients undergoing surgery. Bioactive implants designed with strategic biomimicry, specifically for the application of rotator cuff repair augmentation yielded exciting results and may prove effective in stimulating healing in humans.

Given the opportunity to continue in this field of research, focus on several key areas of interest would be sought. First, investigating supraspinatus muscle

atrophy and fatty infiltration as a prognostic factor for healing would be valuable. A large amount of literature relating to prognostic factors of rotator cuff repair healing suggest these factors may play a role. In addition, conducting a randomised controlled trial investigating the clinical efficacy of current biological and synthetic patches in rotator cuff repair augmentation would be essential before advocacy of their widespread use. Finally, experiments designed to better understand the influence of the electrospun PDO/PCL patch and electrospun PDO suture on the biomechanical properties of the healing tendon, as well as on an enthesis, may lead to valuable insights into how these implants perform *in vivo*.

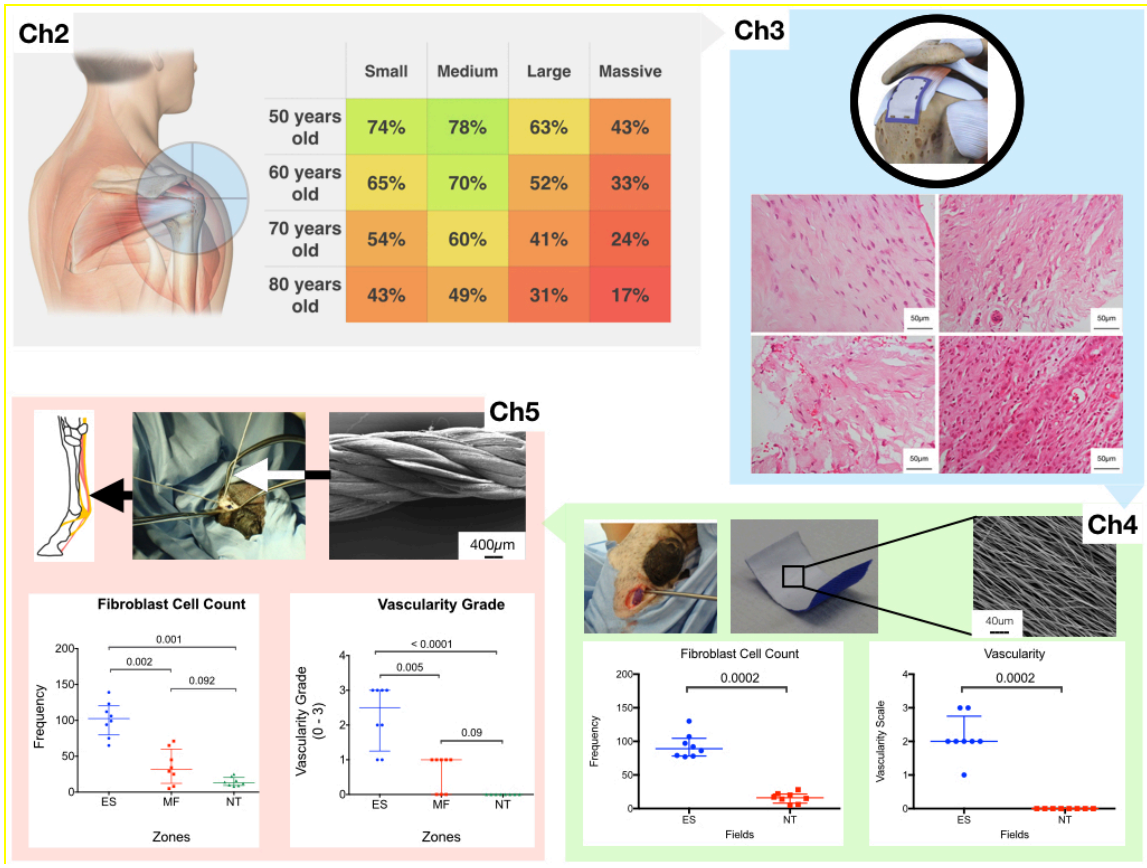


Figure 6-1: Summary of key findings of thesis chapters. Experimental chapters (2-5) are labelled Ch2, Ch3, etc. Chapter 2 findings (top left): shows the predictive model for healing following rotator cuff repair. Chapter 3 findings (top right): shows ECM disruption from histology sections of supraspinatus tendon augmented with GraftJacket, Permacol, or no patch. Chapter 4 findings (bottom right): shows increase in fibroblast cell count and vascularity in response to electrospun PDO/PCL patch implanted in sheep tendons. Chapter 5 findings (bottom left): shows increase in fibroblast cell count and vascularity in response to electrospun PDO suture compared to monofilament PDO suture control.

References

1. McPherson E, Friedman R, An Y, et al. Anthropometric study of normal glenohumeral relationships. *Journal of Shoulder and Elbow Surgery* 1997;6(2):105-12.
2. Lajtai G. Anatomical Shoulder Update 1 - Anatomy Demonstration. In: Latjai G, ed. Anatiomical Knowledge Update: vumedi.com, 2009.
3. Clark J, Harryman D. Tendons, ligaments, and capsule of the rotator cuff. Gross and microscopic anatomy. *J Bone Joint Surg Am* 1992;74A(5):713-25.
4. Burkhart S, Esch J, Jolson R. The rotator crescent and rotator cable: an anatomic description of the shoulder's "suspension bridge". *Arthroscopy* 1993;9(6):611-16.
5. Morag Y, Jacobson J, Lucas D, et al. US appearance of the rotator cable with histologic correlation: preliminary results. *Radiology* 2006;24(2):485-91.
6. Gyftopoulos S, Bencardino J, Nevsky G, et al. Rotator cable: MRI study of its appearance in the intact rotator cuff with anatomic and histologic correlation. *AJR Am J Roentgenol* 2013;200(5):1101-5. doi: 10.2214/AJR.12.9312 [published Online First: 2013/04/27]
7. Taylor D. The life and teaching of William Sharpey (1802-1880) 'Father of Modern Physiology' in Britain. *Med Hist* 1971;15(2):126-53.
8. Benjamin M, Evans EJ, Copp L. The histology of tendon attachments to bone in man. *J Anat* 1986;149:89-100.
9. Rothrauff BB, Pauyo T, Debski RE, et al. The Rotator Cuff Organ: Integrating Developmental Biology, Tissue Engineering, and Surgical Considerations to Treat Chronic Massive Rotator Cuff Tears. *Tissue Eng Part B Rev* 2017;23(4):318-35. doi: 10.1089/ten.TEB.2016.0446 [published Online First: 2017/01/14]
10. Itoi E, Berglund L, Grabowski J, et al. Tensile Properties of the Supraspinatus Tendon. *Journal of Orthopaedic Research* 1995;13(4):578-84.
11. Chaudhury S, Holland C, Vollrath F, et al. Comparing normal and torn rotator cuff
tendons using dynamic shear analysis. *The Journal of Bone and Joint Surgery* 2011;93-B(7):942-48. doi: 10.1302/0301-620X.93B7
12. Wakabayashi I, Itoi E, Sano H, et al. Mechanical environment of the supraspinatus tendon: a two-dimensional finite element model analysis. *Journal of Shoulder and Elbow Surgery* 2003;12(6):612-17. doi: 10.1016/s1058-2746(03)00214-3
13. Yamamoto N, Itoi E. A review of biomechanics of the shoulder and biomechanical concepts of rotator cuff repair. *Asia Pac J Sports Med Arthrosc Rehabil Technol* 2015;2(1):27-30. doi: 10.1016/j.asmart.2014.11.004 [published Online First: 2015/01/17]
14. Bechtol C. Biomechanics of the Shoulder. *Clinical orthopaedics and related research* 1980;146:37-41.
15. Lugo R, Kung P, Ma CB. Shoulder biomechanics. *Eur J Radiol* 2008;68(1):16-24. doi: 10.1016/j.ejrad.2008.02.051 [published Online First: 2008/05/31]

16. Bouaicha S, Slankamenac K, Moor BK, et al. Cross-Sectional Area of the Rotator Cuff Muscles in MRI - Is there Evidence for a Biomechanical Balanced Shoulder? *PLoS One* 2016;11(6):e0157946. doi: 10.1371/journal.pone.0157946 [published Online First: 2016/06/24]
17. Shinozaki N, Sano H, Omi R, et al. Differences in muscle activities during shoulder elevation in patients with symptomatic and asymptomatic rotator cuff tears: analysis by positron emission tomography. *J Shoulder Elbow Surg* 2014;23(3):e61-7. doi: 10.1016/j.jse.2013.06.009 [published Online First: 2013/09/10]
18. Tokish JM, Alexander TC, Kissenberth MJ, et al. Pseudoparalysis: a systematic review of term definitions, treatment approaches, and outcomes of management techniques. *J Shoulder Elbow Surg* 2017;26(6):e177-e87. doi: 10.1016/j.jse.2017.02.024 [published Online First: 2017/05/21]
19. Hawkes DH, Alizadehkhaiyat O, Kemp GJ, et al. Shoulder muscle activation and coordination in patients with a massive rotator cuff tear: an electromyographic study. *J Orthop Res* 2012;30(7):1140-6. doi: 10.1002/jor.22051 [published Online First: 2012/01/04]
20. Yamaguchi K, Sher J, Andersen W, et al. Glenohumeral motion in patients with rotator cuff tears: a comparison of asymptomatic and symptomatic shoulders. *Journal of Shoulder and Elbow Surgery* 2000;9(1):6-11.
21. Minagawa H, Yamamoto N, Abe H, et al. Prevalence of symptomatic and asymptomatic rotator cuff tears in the general population: From mass-screening in one village. *J Orthop* 2013;10(1):8-12. doi: 10.1016/j.jor.2013.01.008
22. Tempelhof S, Rupp S, Seil R. Age-related prevalence of rotator cuff tears in asymptomatic shoulders. *Journal of Shoulder and Elbow Surgery* 1999;8(4):296-99.
23. Hinsley H, Nicholls A, Daines M, et al. Classification of rotator cuff tendinopathy using high definition ultrasound. *Muscles, Ligaments and Tendons Journal* 2014;4(3):391-97.
24. Carr A, Cooper C, Campbell M, et al. Effectiveness of open and arthroscopic rotator cuff repair (UKUFF). A Randomised Controlled Trial. *Bone Joint J* 2017;99-B(1):107-15. doi: 10.1302/0301-620X.99B1
25. Ladermann A, Burkhart SS, Hoffmeyer P, et al. Classification of full-thickness rotator cuff lesions: a review. *EFORT Open Rev* 2016;1(12):420-30. doi: 10.1302/2058-5241.1.160005 [published Online First: 2017/05/04]
26. Gerber C, Krushell R. Isolated rupture of the tendon of the subscapularis muscle. Clinical features in 16 cases. *J Bone Joint Surg Br* 1991;73(3):389-94.
27. Millett P, Warth R. Posterosuperior Rotator Cuff Tears: Classification, Pattern Recognition, and Treatment. *Journal of the American Academy of Orthopaedic Surgeons* 2014;22(8):521-34. doi: 10.5435/
28. Denard PJ, Burkhart SS. Arthroscopic recognition and repair of the torn subscapularis tendon. *Arthrosc Tech* 2013;2(4):e373-9. doi: 10.1016/j.eats.2013.05.007 [published Online First: 2014/01/09]

29. Lo I, Burkhart S. Arthroscopic Repair of Massive, Contracted, Immobile Rotator Cuff Tears Using Single and Double Interval Slides: Technique and Preliminary Results. *Arthroscopy* 2004;20(1):22-33. doi: 10.1016/S0749-8063(03)01112-5
30. Burkhart S, Athanasiou K, Wirth M. Margin Convergence: A Method of Reducing Strain in Massive Rotator Cuff Tears. *Arthroscopy* 1996;12(3):335-38.
31. Jo CH, Shin JS, Lee YG, et al. Platelet-rich plasma for arthroscopic repair of large to massive rotator cuff tears: a randomized, single-blind, parallel-group trial. *Am J Sports Med* 2013;41(10):2240-8. doi: 10.1177/0363546513497925
32. Lapner PL, Sabri E, Rakhra K, et al. A multicenter randomized controlled trial comparing single-row with double-row fixation in arthroscopic rotator cuff repair. *J Bone Joint Surg Am* 2012;94(14):1249-57. doi: 10.2106/JBJS.K.00999 [published Online First: 2012/07/20]
33. Kim JR, Cho YS, Ryu KJ, et al. Clinical and radiographic outcomes after arthroscopic repair of massive rotator cuff tears using a suture bridge technique: assessment of repair integrity on magnetic resonance imaging. *Am J Sports Med* 2012;40(4):786-93. doi: 10.1177/0363546511434546
34. Hayes K, Walton JR, Szomor ZL, et al. Reliability of 3 methods for assessing shoulder strength. *J Shoulder Elbow Surg* 2002;11(1):33-9. doi: 10.1067/mse.2002.119852 [published Online First: 2002/02/15]
35. Hermans J, Luime JJ, Meuffels DE, et al. Does this patient with shoulder pain have rotator cuff disease?: The Rational Clinical Examination systematic review. *JAMA* 2013;310(8):837-47. doi: 10.1001/jama.2013.276187 [published Online First: 2013/08/29]
36. Lenza M, Buchbinder R, Takwoingi Y, et al. Magnetic resonance imaging, magnetic resonance arthrography and ultrasonography for assessing rotator cuff tears in people with shoulder pain for whom surgery is being considered. *Cochrane Database Syst Rev* 2013(9):CD009020. doi: 10.1002/14651858.CD009020.pub2 [published Online First: 2013/09/26]
37. Jeong JY, Min SK, Park KM, et al. Location of Rotator Cuff Tear Initiation: A Magnetic Resonance Imaging Study of 191 Shoulders. *Am J Sports Med* 2018;46(3):649-55. doi: 10.1177/0363546517748925 [published Online First: 2018/01/10]
38. Determe D, Rongieres M, Kany J, et al. Anatomic study of the tendinous rotator cuff of the shoulder. *Surg Radiol Anat* 1996;18:195-200.
39. Ling S, Chen C, Wan R. A study on the vascular supply of the supraspinatus tendon. *Surg Radiol Anat* 1990;12:161-65.
40. Hegedus EJ, Cook C, Brennan M, et al. Vascularity and tendon pathology in the rotator cuff: a review of literature and implications for rehabilitation and surgery. *Br J Sports Med* 2010;44(12):838-47. doi: 10.1136/bjsm.2008.053769 [published Online First: 2009/03/19]
41. Matthews T, Hand G, Rees J, et al. Pathology of the torn rotator cuff tendon. *J Bone Joint Surg [Br]* 2006;88-B(4):489-95. doi: 10.1302/0301-620X.88B4
42. Neer C. Impingement Lesions. *Clin Orthop Relat Res* 1983(173):70-77.

43. Nicholson G, Goodman D, Flatow E, et al. The acromion: Morphologic condition and age-related changes. A study of 420 scapulas. 1996;5(1):1-11.
44. Moor B, Bouaicha S, Rothenfluh D, et al. Is there an association between the individual anatomy of the scapula and the development of rotator cuff tears or osteoarthritis of the glenohumeral joint? A Radiological Study Of The Critical Shoulder Angle. *Bone Joint J* 2013;95-B(7):935-41. doi: 10.1302/0301-620X.95B7
45. Garcia GH, Liu JN, Degen RM, et al. Higher critical shoulder angle increases the risk of retear after rotator cuff repair. *J Shoulder Elbow Surg* 2016 doi: 10.1016/j.jse.2016.07.009
46. Abrams G, Gupta A, Hussey K, et al. Arthroscopic Repair of Full-Thickness Rotator Cuff Tears With and Without Acromioplasty: Randomized Prospective Trial With 2-Year Follow-up. *Am J Sports Med* 2014;42(6):1296-303. doi: 10.1177/0363546514529091 [published Online First: 2014/04/16]
47. MacDonald P, McRae S, Leiter J, et al. Arthroscopic rotator cuff repair with and without acromioplasty in the treatment of full-thickness rotator cuff tears: a multicenter, randomized controlled trial. *J Bone Joint Surg Am* 2011;93(21):1953-60. doi: 10.2106/JBJS.K.00488 [published Online First: 2011/11/04]
48. Abrams G, Luria A, Carr R, et al. Association of synovial inflammation and inflammatory mediators with glenohumeral rotator cuff pathology. *J Shoulder Elbow Surg* 2016;25(6):989-97. doi: 10.1016/j.jse.2015.10.011
49. Fabis J, Szemraj J, Strek M, et al. Is resection of the tendon edge necessary to enhance the healing process? An evaluation of the expression of collagen type I, IL-1beta, IFN-gamma, IL-4, and IL-13 in the distal 1 cm of a torn supraspinatus tendon: part II. *J Shoulder Elbow Surg* 2014;23(12):1779-85. doi: 10.1016/j.jse.2014.08.023
50. Dakin SG, Martinez FO, Yapp C, et al. Inflammation activation and resolution in human tendon disease. *Sci Transl Med* 2015;7(311):311ra173. doi: 10.1126/scitranslmed.aac4269
51. Moosmayer S, Tariq R, Stiris M, et al. The natural history of asymptomatic rotator cuff tears: a three-year follow-up of fifty cases. *J Bone Joint Surg Am* 2013;95(14):1249-55. doi: 10.2106/JBJS.L.00185 [published Online First: 2013/07/19]
52. Yamaguchi K, Tetro AM, Blam O, et al. Natural history of asymptomatic rotator cuff tears: a longitudinal analysis of asymptomatic tears detected sonographically. *J Shoulder Elbow Surg* 2001;10(3):199-203. doi: 10.1067/mse.2001.113086
53. Mall NA, Kim HM, Keener JD, et al. Symptomatic progression of asymptomatic rotator cuff tears: a prospective study of clinical and sonographic variables. *J Bone Joint Surg Am* 2010;92(16):2623-33. doi: 10.2106/JBJS.I.00506 [published Online First: 2010/11/19]
54. Fucentese SF, von Roll AL, Pfirrmann CW, et al. Evolution of nonoperatively treated symptomatic isolated full-thickness supraspinatus tears. *J Bone Joint Surg Am* 2012;94(9):801-8. doi: 10.2106/JBJS.I.01286 [published Online First: 2012/05/04]

55. Safran O, Schroeder J, Bloom R, et al. Natural history of nonoperatively treated symptomatic rotator cuff tears in patients 60 years old or younger. *Am J Sports Med* 2011;39(4):710-4. doi: 10.1177/0363546510393944 [published Online First: 2011/02/12]
56. Yamamoto N, Mineta M, Kawakami J, et al. Risk Factors for Tear Progression in Symptomatic Rotator Cuff Tears: A Prospective Study of 174 Shoulders. *Am J Sports Med* 2017;45(11):2524-31. doi: 10.1177/0363546517709780 [published Online First: 2017/06/14]
57. Nakamura Y, Yokoya S, Mochizuki Y, et al. Monitoring of progression of nonsurgically treated rotator cuff tears by magnetic resonance imaging. *J Orthop Sci* 2015;20(2):314-20. doi: 10.1007/s00776-014-0680-6 [published Online First: 2014/12/30]
58. Kong BY, Cho M, Lee HR, et al. Structural Evolution of Nonoperatively Treated High-Grade Partial-Thickness Tears of the Supraspinatus Tendon. *Am J Sports Med* 2018;46(1):79-86. doi: 10.1177/0363546517729164 [published Online First: 2017/09/28]
59. Colvin AC, Egorova N, Harrison AK, et al. National trends in rotator cuff repair. *J Bone Joint Surg Am* 2012;94(3):227-33. doi: 10.2106/JBJS.J.00739 [published Online First: 2012/02/03]
60. Jain NB, Higgins LD, Losina E, et al. Epidemiology of musculoskeletal upper extremity ambulatory surgery in the United States. *BMC Musculoskelet Disord* 2014;15:4. doi: 10.1186/1471-2474-15-4
61. Digital N. Hospital Admitted Patient Care Activity, 2015-2016 [NS] http://content.digital.nhs.uk/article/2021/Website-Search?productid=23488&q=title%3a%22Hospital+Episode+Statistics%2c+Admitted+patient+care+-+England%22+or+title%3a%22Hospital+Admitted+Patient+Care+Activity%22&sort=Relevance&size=10&page=1&area=both - top: NHS Digital; 2016 [accessed 03/08/2017 2017.
62. Randelli P, Cucchi D, Ragone V, et al. History of rotator cuff surgery. *Knee Surg Sports Traumatol Arthrosc* 2015;23(2):344-62. doi: 10.1007/s00167-014-3445-z
63. Codman E. The Shoulder: Thomas Todd Company 1934.
64. Debeyre J PD, Elmelik E. Repair of ruptures of the rotator cuff of the shoulder. *Journal of Bone and Joint Surgery* 1965;47-B(1):36-42.
65. Burman M. Arthroscopy or the Driect Visualization of Joints. *Journal of Bone and Joint Surgery* 1931;13(4):669-95.
66. Burkhart SS, Denard PJ, Konicek J, et al. Biomechanical validation of load-sharing rip-stop fixation for the repair of tissue-deficient rotator cuff tears. *Am J Sports Med* 2014;42(2):457-62. doi: 10.1177/0363546513516602 [published Online First: 2014/01/01]
67. Denard PJ, Burkhart SS. A load-sharing rip-stop fixation construct for arthroscopic rotator cuff repair. *Arthrosc Tech* 2012;1(1):e37-42. doi: 10.1016/j.eats.2011.12.005 [published Online First: 2013/06/15]
68. Park MC, Peterson AB, McGarry MH, et al. Knotless Transosseous-Equivalent Rotator Cuff Repair Improves Biomechanical Self-reinforcement Without Diminishing Footprint Contact Compared With

Medial Knotted Repair. *Arthroscopy* 2017;33(8):1473-81. doi: 10.1016/j.arthro.2017.03.021 [published Online First: 2017/07/08]
69. Maguire M, Goldberg J, Bokor D, et al. Biomechanical evaluation of four different transosseous-equivalent/suture bridge rotator cuff repairs. *Knee Surg Sports Traumatol Arthrosc* 2011;19(9):1582-7. doi: 10.1007/s00167-011-1436-x [published Online First: 2011/02/25]
70. Leek BT, Robertson C, Mahar A, et al. Comparison of mechanical stability in double-row rotator cuff repairs between a knotless transtendon construct versus the addition of medial knots. *Arthroscopy* 2010;26(9 Suppl):S127-33. doi: 10.1016/j.arthro.2010.02.035 [published Online First: 2010/09/10]
71. Lorbach O, Bachelier F, Vees J, et al. Cyclic loading of rotator cuff reconstructions: single-row repair with modified suture configurations versus double-row repair. *Am J Sports Med* 2008;36(8):1504-10. doi: 10.1177/0363546508314424 [published Online First: 2008/02/26]
72. Zheng N, Harris HW, Andrews JR. Failure analysis of rotator cuff repair: a comparison of three double-row techniques. *J Bone Joint Surg Am* 2008;90(5):1034-42. doi: 10.2106/JBJS.G.00049 [published Online First: 2008/05/03]
73. Chu T, McDonald E, Tufaga M, et al. Comparison of completely knotless and hybrid double-row fixation systems: a biomechanical study. *Arthroscopy* 2011;27(4):479-85. doi: 10.1016/j.arthro.2010.09.015 [published Online First: 2011/02/01]
74. Demirhan M, Atalar AC, Kilicoglu O. Primary fixation strength of rotator cuff repair techniques: a comparative study. *Arthroscopy: The Journal of Arthroscopic & Related Surgery* 2003;19(6):572-76. doi: 10.1016/s0749-8063(03)00126-9
75. Kullar RS, Reagan JM, Kolz CW, et al. Suture placement near the musculotendinous junction in the supraspinatus: implications for rotator cuff repair. *Am J Sports Med* 2015;43(1):57-62. doi: 10.1177/0363546514553091 [published Online First: 2014/10/25]
76. Cole BJ, ElAttrache NS, Anbari A. Arthroscopic rotator cuff repairs: an anatomic and biomechanical rationale for different suture-anchor repair configurations. *Arthroscopy* 2007;23(6):662-9. doi: 10.1016/j.arthro.2007.02.018 [published Online First: 2007/06/15]
77. Park MC, ElAttrache NS, Tibone JE, et al. Part I: Footprint contact characteristics for a transosseous-equivalent rotator cuff repair technique compared with a double-row repair technique. *J Shoulder Elbow Surg* 2007;16(4):461-8. doi: 10.1016/j.jse.2006.09.010 [published Online First: 2007/02/27]
78. Smith GCS, Bouwmeester TM, Lam PH. Knotless double-row SutureBridge rotator cuff repairs have improved self-reinforcement compared with double-row SutureBridge repairs with tied medial knots: a biomechanical study using an ovine model. *J Shoulder Elbow Surg* 2017;26(12):2206-12. doi: 10.1016/j.jse.2017.06.045 [published Online First: 2017/09/25]
79. Wieser K, Farshad M, Vlachopoulos L, et al. Suture slippage in knotless suture anchors as a potential failure mechanism in rotator cuff repair. *Arthroscopy* 2012;28(11):1622-7. doi: 10.1016/j.arthro.2012.04.150 [published Online First: 2012/09/18]

80. Rhee YG, Cho NS, Parke CS. Arthroscopic rotator cuff repair using modified Mason-Allen medial row stitch: knotless versus knot-tying suture bridge technique. *Am J Sports Med* 2012;40(11):2440-7. doi: 10.1177/0363546512459170 [published Online First: 2012/09/25]
81. Development" ARa. White Paper: Histological Analysis of the Vented BioComposite SwiveLock in the Canine Humerus. https://d1psc3qesfsa61.cloudfront.net/pdfs/sjjb6fkEEeCRTQBQVoRHOw/sjjb6fkEEeCRTQBQVoRHOw.pdf?Expires=1521389362&Signature=DofE5eAwINkJC6TJZ755tOfgErBqI3HgqcgLjna49scvz37Z1FzcBDwXqANsKBMLd1YJF-JvM9z0o9SRr%7EvLZO8P21jryRvUXrhoUUep3IHXI5vNKfHMjauz0JRQVgDZu3pF4uHjnY8StkEjw8Q34uCnUcHOM0-0ooQz-snjDkLufj88RPIMGNYqc9cEFuzHR2s2EPZZWEKoV1xgn4%7Eo5EcuP3euevQB1RcaI8kTEVqaJSLPxPWDPekv-Q80%7ETYdcQt0DCDPMFBbKLSUxV-uOwf-UKEEgYd4344HyJHes4cLeu3Gen-9Xq8e9WjUDT%7EoBZg2T7Wjl24rvzVdiGYtGg__&Key-Pair-Id=APKAJMGJRW6JX5OBM5LA: Arthex Inc., 2010:1.
82. Nagra NS, Zargar N, Smith RDJ, et al. Mechanical properties of all-suture anchors for rotator cuff repair. *Bone Joint Res* 2017;6(2):82-89. doi: 10.1302/2046-3758.62.bJr-
83. Bisson LJ, Manohar LM. A biomechanical comparison of the pullout strength of No. 2 FiberWire suture and 2-mm FiberWire tape in bovine rotator cuff tendons. *Arthroscopy* 2010;26(11):1463-8. doi: 10.1016/j.arthro.2010.04.075 [published Online First: 2010/10/06]
84. Barber F, Drew O. A biomechanical comparison of tendon-bone interface motion and cyclic loading between single-row, triple-loaded cuff repairs and double-row, suture-tape cuff repairs using biocomposite anchors. *Arthroscopy* 2012;28(9):1197-205. doi: 10.1016/j.arthro.2012.02.015 [published Online First: 2012/05/18]
85. Deranlot J, Maurel N, Diop A, et al. Abrasive properties of braided polyblend sutures in cuff tendon repair: an in vitro biomechanical study exploring regular and tape sutures. *Arthroscopy* 2014;30(12):1569-73. doi: 10.1016/j.arthro.2014.06.018 [published Online First: 2014/08/26]
86. Arthrex. SutureTape https://www.arthrex.com/shoulder/suturetape: Arthrex; 2018 [accessed 09/03/2018 2018.
87. Kirkley A, Griffin S, Dainty K. Scoring Systems for the Functional Assessment of the Shoulder. *Arthroscopy* 2003;19(10):1109-20. doi: 10.1016/S0749-8063(03)00973-3
88. Schmidt S, Ferrer M, Gonzalez M, et al. Evaluation of shoulder-specific patient-reported outcome measures: a systematic and standardized comparison of available evidence. *J Shoulder Elbow Surg* 2014;23(3):434-44. doi: 10.1016/j.jse.2013.09.029 [published Online First: 2014/01/11]
89. Dawson J, Rogers K, Fitzpatrick R, et al. The Oxford shoulder score revisited. *Arch Orthop Trauma Surg* 2009;129(1):119-23. doi: 10.1007/s00402-007-0549-7 [published Online First: 2008/01/10]
90. Colliver J, Wang A, Joss B, et al. Early postoperative repair status after rotator cuff repair cannot be accurately classified using questionnaires of patient function and isokinetic strength evaluation. *J Shoulder Elbow Surg*

2016;25(4):536-42. doi: 10.1016/j.jse.2015.09.019 [published Online First: 2015/12/22]
91. Sugaya H, Maeda K, Matsuki K, et al. Functional and structural outcome after arthroscopic full-thickness rotator cuff repair: single-row versus dual-row fixation. *Arthroscopy* 2005;21(11):1307-16. doi: 10.1016/j.arthro.2005.08.011 [published Online First: 2005/12/06]
92. Niglis L, Collin P, Dosch JC, et al. Intra- and inter-observer agreement in MRI assessment of rotator cuff healing using the Sugaya classification 10years after surgery. *Orthop Traumatol Surg Res* 2017;103(6):835-39. doi: 10.1016/j.otsr.2017.06.006 [published Online First: 2017/06/29]
93. Kennedy JS, Myers HS, Gibson SD, et al. The validity and reliability of ultrasound on identifying supraspinatus tears during passive external rotation from 0 degrees to 30 degrees : a pilot project. *Shoulder Elbow* 2017;9(1):54-60. doi: 10.1177/1758573216655079 [published Online First: 2017/06/03]
94. Oh JH, Kim SH, Ji HM, et al. Prognostic factors affecting anatomic outcome of rotator cuff repair and correlation with functional outcome. *Arthroscopy* 2009;25(1):30-9. doi: 10.1016/j.arthro.2008.08.010 [published Online First: 2008/12/30]
95. Charousset C, Grimberg J, Duranthon LD, et al. The time for functional recovery after arthroscopic rotator cuff repair: correlation with tendon healing controlled by computed tomography arthrography. *Arthroscopy* 2008;24(1):25-33. doi: 10.1016/j.arthro.2007.07.023 [published Online First: 2008/01/10]
96. Flurin P-H, Landreau P, Gregory T, et al. Cuff Integrity After Arthroscopic Rotator Cuff Repair: Correlation With Clinical Results in 576 Cases. *Arthroscopy: The Journal of Arthroscopic & Related Surgery* 2007;23(4):340-46. doi: 10.1016/j.arthro.2007.01.005
97. Lafosse L, Brozska R, Toussaint B, et al. The outcome and structural integrity of arthroscopic rotator cuff repair with use of the double-row suture anchor technique. *J Bone Joint Surg Am* 2007;89(7):1533-41. doi: 10.2106/JBJS.F.00305 [published Online First: 2007/07/04]
98. Boileau P, Brassart N, Watkinson D, et al. Arthroscopic Repair of Full-Thickness Tears of the Supraspinatus: Does the Tendon Really Heal? *Journal of Bone & Joint Surgery (Am)* 2005;87A(6):1229-40.
99. Park JG, Cho NS, Song JH, et al. Rotator Cuff Repair in Patients over 75 Years of Age: Clinical Outcome and Repair Integrity. *Clin Orthop Surg* 2016;8(4):420-27. doi: 10.4055/cios.2016.8.4.420 [published Online First: 2016/12/03]
100. Russell RD, Knight JR, Mulligan E, et al. Structural integrity after rotator cuff repair does not correlate with patient function and pain: a meta-analysis. *J Bone Joint Surg Am* 2014;96(4):265-71. doi: 10.2106/JBJS.M.00265
101. Kim HM, Caldwell JM, Buza JA, et al. Factors affecting satisfaction and shoulder function in patients with a recurrent rotator cuff tear. *J Bone Joint Surg Am* 2014;96(2):106-12. doi: 10.2106/JBJS.L.01649 [published Online First: 2014/01/17]
102. Lafosse L, Brzoska R, Toussaint B, et al. The outcome and structural integrity of arthroscopic rotator cuff repair with use of the double-row

suture anchor technique. Surgical technique. *J Bone Joint Surg Am* 2008;90 Suppl 2 Pt 2:275-86. doi: 10.2106/JBJS.H.00388
103. Maqdes A, Abarca J, Moraiti C, et al. Does preoperative subscapularis fatty muscle infiltration really matter in anterosuperior rotator cuff tears repair outcomes? A prospective multicentric study. *Orthop Traumatol Surg Res* 2014;100(5):485-8. doi: 10.1016/j.otsr.2014.02.010
104. Galatz LM, Ball CM, Teefey SA, et al. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am* 2004;86-A(2):219-24.
105. Antuña S, Barco R, Martínez Díez J, et al. Platelet-rich fibrin in arthroscopic repair of massive rotator cuff tears: A prospective randomized pilot clinical trial. *Acta Orthop Belg* 2013;79(1):25-20.
106. Barber F, Burns J, Deutsch A, et al. A prospective, randomized evaluation of acellular human dermal matrix augmentation for arthroscopic rotator cuff repair. *Arthroscopy* 2012;28(1):8-15. doi: 10.1016/j.arthro.2011.06.038 [published Online First: 2011/10/08]
107. Ma HL, Chiang ER, Wu HT, et al. Clinical outcome and imaging of arthroscopic single-row and double-row rotator cuff repair: a prospective randomized trial. *Arthroscopy* 2012;28(1):16-24. doi: 10.1016/j.arthro.2011.07.003
108. Lee BG, Cho NS, Rhee YG. Effect of two rehabilitation protocols on range of motion and healing rates after arthroscopic rotator cuff repair: aggressive versus limited early passive exercises. *Arthroscopy* 2012;28(1):34-42. doi: 10.1016/j.arthro.2011.07.012
109. Cuff DJ, Pupello DR. Prospective randomized study of arthroscopic rotator cuff repair using an early versus delayed postoperative physical therapy protocol. *J Shoulder Elbow Surg* 2012;21(11):1450-5. doi: 10.1016/j.jse.2012.01.025
110. Gartsman GM, Drake G, Edwards TB, et al. Ultrasound evaluation of arthroscopic full-thickness supraspinatus rotator cuff repair: single-row versus double-row suture bridge (transosseous equivalent) fixation. Results of a prospective, randomized study. *J Shoulder Elbow Surg* 2013;22(11):1480-7. doi: 10.1016/j.jse.2013.06.020 [published Online First: 2013/09/10]
111. Arndt J, Clavert P, Mielcarek P, et al. Immediate passive motion versus immobilization after endoscopic supraspinatus tendon repair: a prospective randomized study. *Orthop Traumatol Surg Res* 2012;98(6 Suppl):S131-8. doi: 10.1016/j.otsr.2012.05.003
112. Ko SH, Lee CC, Friedman D, et al. Arthroscopic single-row supraspinatus tendon repair with a modified mattress locking stitch: a prospective, randomized controlled comparison with a simple stitch. *Arthroscopy* 2008;24(9):1005-12. doi: 10.1016/j.arthro.2008.04.074
113. Charousset C, Zaoui A, Bellaiche L, et al. Does autologous leukocyte-platelet-rich plasma improve tendon healing in arthroscopic repair of large or massive rotator cuff tears? *Arthroscopy* 2014;30(4):428-35. doi: 10.1016/j.arthro.2013.12.018
114. Gilot GJ, Alvarez-Pinzon AM, Barcksdale L, et al. Outcome of Large to Massive Rotator Cuff Tears Repaired With and Without Extracellular

Matrix Augmentation: A Prospective Comparative Study. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association* 2015;31(8):1459-65. doi: http://dx.doi.org/10.1016/j.arthro.2015.02.032

115. Iannotti JP, Codsi MJ, Kwon YW, et al. Porcine small intestine submucosa augmentation of surgical repair of chronic two-tendon rotator cuff tears. A randomized, controlled trial. *J Bone Joint Surg Am* 2006;88(6):1238-44. doi: 10.2106/JBJS.E.00524
116. Boyer P, Bouthors C, Delcourt T, et al. Arthroscopic double-row cuff repair with suture-bridging: a structural and functional comparison of two techniques. *Knee Surg Sports Traumatol Arthrosc* 2015;23(2):478-86. doi: 10.1007/s00167-013-2401-7
117. Hernigou P, Flouzat Lachaniette CH, Delambre J, et al. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: A case-controlled study. *International Orthopaedics* 2014;38(9):1811-18. doi: http://dx.doi.org/10.1007/s00264-014-2391-1
118. Ciampi P, Scotti C, Nonis A, et al. The benefit of synthetic versus biological patch augmentation in the repair of posterosuperior massive rotator cuff tears: a 3-year follow-up study. *The American journal of sports medicine* 2014;42(5):1169-75.
119. Cho NS, Moon SC, Jeon JW, et al. The influence of diabetes mellitus on clinical and structural outcomes after arthroscopic rotator cuff repair. *Am J Sports Med* 2015;43(4):991-7. doi: 10.1177/0363546514565097
120. Tudisco C, Bisicchia S, Savarese E, et al. Single-row vs. double-row arthroscopic rotator cuff repair: clinical and 3 Tesla MR arthrography results. *BMC Musculoskelet Disord* 2013;14:43. doi: 10.1186/1471-2474-14-43
121. Wang E, Wang L, Gao P, et al. Single-versus double-row arthroscopic rotator cuff repair in massive tears. *Medical science monitor : international medical journal of experimental and clinical research* 2015;21:1556-61. doi: 10.12659/MSM.893058
122. Rhee YG, Cho NS, Yoo JH. Clinical outcome and repair integrity after rotator cuff repair in patients older than 70 years versus patients younger than 70 years. *Arthroscopy* 2014;30(5):546-54. doi: 10.1016/j.arthro.2014.02.006
123. Robertson CM, Chen CT, Shindle MK, et al. Failed healing of rotator cuff repair correlates with altered collagenase and gelatinase in supraspinatus and subscapularis tendons. *Am J Sports Med* 2012;40(9):1993-2001. doi: 10.1177/0363546512456519
124. Park JS, Park HJ, Kim SH, et al. Prognostic Factors Affecting Rotator Cuff Healing After Arthroscopic Repair in Small to Medium-sized Tears. *Am J Sports Med* 2015;43(10):2386-92. doi: 10.1177/0363546515594449
125. Lichtenberg S, Liem D, Magosch P, et al. Influence of tendon healing after arthroscopic rotator cuff repair on clinical outcome using single-row Mason-Allen suture technique: a prospective, MRI controlled study. *Knee*

Surg Sports Traumatol Arthrosc 2006;14(11):1200-6. doi: 10.1007/s00167-006-0132-8
126. Meyer M, Klouche S, Rousselin B, et al. Does arthroscopic rotator cuff repair actually heal? Anatomic evaluation with magnetic resonance arthrography at minimum 2 years follow-up. *J Shoulder Elbow Surg* 2012;21(4):531-6. doi: 10.1016/j.jse.2011.02.009
127. Chung SW, Oh JH, Gong HS, et al. Factors affecting rotator cuff healing after arthroscopic repair: osteoporosis as one of the independent risk factors. *Am J Sports Med* 2011;39(10):2099-107. doi: 10.1177/0363546511415659
128. Kerr J, Borbas P, Meyer DC, et al. Arthroscopic rotator cuff repair in the weight-bearing shoulder. *J Shoulder Elbow Surg* 2015;24(12):1894-9. doi: 10.1016/j.jse.2015.05.051
129. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928. doi: 10.1136/bmj.d5928 [published Online First: 2011/10/20]
130. Castricini R, Longo UG, De Benedetto M, et al. Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomized controlled trial. *Am J Sports Med* 2011;39(2):258-65. doi: 10.1177/0363546510390780
131. Milano G, Saccomanno MF, Careri S, et al. Efficacy of marrow-stimulating technique in arthroscopic rotator cuff repair: a prospective randomized study. *Arthroscopy* 2013;29(5):802-10. doi: 10.1016/j.arthro.2013.01.019
132. Malavolta EA, Gracitelli ME, Ferreira Neto AA, et al. Platelet-rich plasma in rotator cuff repair: a prospective randomized study. *Am J Sports Med* 2014;42(10):2446-54. doi: 10.1177/0363546514541777
133. Franceschi F, Ruzzini L, Longo UG, et al. Equivalent clinical results of arthroscopic single-row and double-row suture anchor repair for rotator cuff tears: a randomized controlled trial. *Am J Sports Med* 2007;35(8):1254-60. doi: 10.1177/0363546507302218
134. Koh KH, Kang KC, Lim TK, et al. Prospective randomized clinical trial of single- versus double-row suture anchor repair in 2- to 4-cm rotator cuff tears: clinical and magnetic resonance imaging results. *Arthroscopy* 2011;27(4):453-62. doi: 10.1016/j.arthro.2010.11.059
135. Kukkonen J, Joukainen A, Lehtinen J, et al. Treatment of Nontraumatic Rotator Cuff Tears: A Randomized Controlled Trial with Two Years of Clinical and Imaging Follow-up. *J Bone Joint Surg Am* 2015;97(21):1729-37. doi: 10.2106/JBJS.N.01051 [published Online First: 2015/11/06]
136. Moosmayer S, Lund G, Seljom U, et al. Comparison between surgery and physiotherapy in the treatment of small and medium-sized tears of the rotator cuff: A randomised controlled study of 103 patients with one-year follow-up. *The Journal of bone and joint surgery British volume* 2010;92(1):83-91. doi: 10.1302/0301-620X.92B1.22609
137. Ruiz-Moneo P, Molano-Munoz J, Prieto E, et al. Plasma rich in growth factors in arthroscopic rotator cuff repair: a randomized, double-blind, controlled clinical trial. *Arthroscopy* 2013;29(1):2-9. doi: 10.1016/j.arthro.2012.08.014

138. Keener JD, Galatz LM, Stobbs-Cucchi G, et al. Rehabilitation following arthroscopic rotator cuff repair: a prospective randomized trial of immobilization compared with early motion. *J Bone Joint Surg Am* 2014;96(1):11-9. doi: 10.2106/JBJS.M.00034
139. Burks RT, Crim J, Brown N, et al. A prospective randomized clinical trial comparing arthroscopic single- and double-row rotator cuff repair: magnetic resonance imaging and early clinical evaluation. *Am J Sports Med* 2009;37(4):674-82. doi: 10.1177/0363546508328115
140. Zumstein MA, Rumian A, Lesbats V, et al. Increased vascularization during early healing after biologic augmentation in repair of chronic rotator cuff tears using autologous leukocyte- and platelet-rich fibrin (L-PRF): A prospective randomized controlled pilot trial. *Journal of Shoulder and Elbow Surgery* 2014;23(1):3-12. doi: http://dx.doi.org/10.1016/j.jse.2013.08.017
141. Gumina S, Campagna V, Ferrazza G, et al. Use of platelet-leukocyte membrane in arthroscopic repair of large rotator cuff tears: a prospective randomized study. *J Bone Joint Surg Am* 2012;94(15):1345-52. doi: 10.2106/JBJS.K.00394
142. Randelli P, Arrigoni P, Ragone V, et al. Platelet rich plasma in arthroscopic rotator cuff repair: a prospective RCT study, 2-year follow-up. *J Shoulder Elbow Surg* 2011;20(4):518-28. doi: 10.1016/j.jse.2011.02.008
143. Jo CH, Shin JS, Shin WH, et al. Platelet-rich plasma for arthroscopic repair of medium to large rotator cuff tears: a randomized controlled trial. *Am J Sports Med* 2015;43(9):2102-10. doi: 10.1177/0363546515587081
144. Carbonel I, Martinez AA, Calvo A, et al. Single-row versus double-row arthroscopic repair in the treatment of rotator cuff tears: a prospective randomized clinical study. *Int Orthop* 2012;36(9):1877-83. doi: 10.1007/s00264-012-1559-9
145. Moosmayer S, Lund G, Seljom US, et al. Tendon repair compared with physiotherapy in the treatment of rotator cuff tears: a randomized controlled study in 103 cases with a five-year follow-up. *J Bone Joint Surg Am* 2014;96(18):1504-14. doi: 10.2106/JBJS.M.01393
146. Kim YS, Chung SW, Kim JY, et al. Is early passive motion exercise necessary after arthroscopic rotator cuff repair? *Am J Sports Med* 2012;40(4):815-21. doi: 10.1177/0363546511434287
147. Rodeo SA, Delos D, Williams RJ, et al. The effect of platelet-rich fibrin matrix on rotator cuff tendon healing: a prospective, randomized clinical study. *Am J Sports Med* 2012;40(6):1234-41. doi: 10.1177/0363546512442924
148. Weber SC, Kauffman JI, Parise C, et al. Platelet-rich fibrin matrix in the management of arthroscopic repair of the rotator cuff: a prospective, randomized, double-blinded study. *The American journal of sports medicine* 2013;41(2):263-70.
149. Moraes VY, Lenza M, Tamaoki MJ, et al. Platelet-rich therapies for musculoskeletal soft tissue injuries. *Cochrane Database Syst Rev* 2014(4):CD010071. doi: 10.1002/14651858.CD010071.pub3 [published Online First: 2014/05/02]

150. Fitzpatrick J, Bulsara MK, McCrory PR, et al. Analysis of Platelet-Rich Plasma Extraction: Variations in Platelet and Blood Components Between 4 Common Commercial Kits. *Orthop J Sports Med* 2017;5(1):2325967116675272. doi: 10.1177/2325967116675272 [published Online First: 2017/02/18]
151. Cho HS, Song IH, Park SY, et al. Individual variation in growth factor concentrations in platelet-rich plasma and its influence on human mesenchymal stem cells. *Korean J Lab Med* 2011;31(3):212-8. doi: 10.3343/kjlm.2011.31.3.212 [published Online First: 2011/07/23]
152. Beitzel K, Chowaniec DM, McCarthy MB, et al. Stability of double-row rotator cuff repair is not adversely affected by scaffold interposition between tendon and bone. *Am J Sports Med* 2012;40(5):1148-54. doi: 10.1177/0363546512437835 [published Online First: 2012/03/01]
153. Hirahara AM, Adams CR. Arthroscopic Superior Capsular Reconstruction for Treatment of Massive Irreparable Rotator Cuff Tears. *Arthrosc Tech* 2015;4(6):e637-41. doi: 10.1016/j.eats.2015.07.006 [published Online First: 2016/02/13]
154. Milks RA, Kolmodin JD, Ricchetti ET, et al. Augmentation with a reinforced acellular fascia lata strip graft limits cyclic gapping of supraspinatus repairs in a human cadaveric model. *J Shoulder Elbow Surg* 2018 doi: 10.1016/j.jse.2017.12.016 [published Online First: 2018/02/06]
155. Veen EJD, Stevens M, Diercks RL. Biceps Autograft Augmentation for Rotator Cuff Repair: A Systematic Review. *Arthroscopy* 2018;34(4):1297-305. doi: 10.1016/j.arthro.2017.10.044 [published Online First: 2018/01/27]
156. Mitchell IC, Garcia NM, Barber R, et al. Permacol: a potential biologic patch alternative in congenital diaphragmatic hernia repair. *J Pediatr Surg* 2008;43(12):2161-4. doi: 10.1016/j.jpedsurg.2008.08.040 [published Online First: 2008/12/02]
157. Martin B, Sangalang M, Wu S, et al. Outcomes of allogenic acellular matrix therapy in treatment of diabetic foot wounds: an initial experience. *International Wound Journal* 2005;2(2):128-39.
158. (FDA) UFDA. 510(k) Premarket Notification. PERMACOL SURGICAL IMPLANT (K043366) https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=K043662004 [2017.
159. Walton JR, Bowman NK, Khatib Y, et al. Restore orthobiologic implant: not recommended for augmentation of rotator cuff repairs. *J Bone Joint Surg Am* 2007;89(4):786-91. doi: 10.2106/JBJS.F.00315 [published Online First: 2007/04/04]
160. "Neoligaments". Leeds-Kuff patch https://www.neoligaments.com/product/leeds-kuff-patch/2017 [accessed 11/01/2018 2018.
161. Flury M, Rickenbacher D, Jung C, et al. Porcine Dermis Patch Augmentation of Supraspinatus Tendon Repairs: A Pilot Study Assessing Tendon Integrity and Shoulder Function 2 Years After Arthroscopic Repair in Patients Aged 60 Years or Older. *Arthroscopy* 2018;34(1):24-37. doi: 10.1016/j.arthro.2017.06.024 [published Online First: 2017/08/22]

162. Consigliere P, Polyzois I, Sarkhel T, et al. Preliminary Results of a Consecutive Series of Large & Massive Rotator Cuff Tears Treated with Arthroscopic Rotator Cuff Repairs Augmented with Extracellular Matrix. *Arch Bone Jt Surg* 2017;5(1)
163. Yoon JP, Chung SW, Kim JY, et al. Outcomes of Combined Bone Marrow Stimulation and Patch Augmentation for Massive Rotator Cuff Tears. *Am J Sports Med* 2016;44(4):963-71. doi: 10.1177/0363546515625044 [published Online First: 2016/02/07]
164. Petri M, Warth RJ, Horan MP, et al. Outcomes After Open Revision Repair of Massive Rotator Cuff Tears With Biologic Patch Augmentation. *Arthroscopy* 2016;32(9):1752-60. doi: 10.1016/j.arthro.2016.01.037 [published Online First: 2016/04/12]
165. Lederman ES, Toth AP, Nicholson GP, et al. A prospective, multicenter study to evaluate clinical and radiographic outcomes in primary rotator cuff repair reinforced with a xenograft dermal matrix. *J Shoulder Elbow Surg* 2016;25(12):1961-70. doi: 10.1016/j.jse.2016.02.029
166. Bryant D, Holtby R, Willits K, et al. A randomized clinical trial to compare the effectiveness of rotator cuff repair with or without augmentation using porcine small intestine submucosa for patients with moderate to large rotator cuff tears: a pilot study. *J Shoulder Elbow Surg* 2016;25(10):1623-33. doi: 10.1016/j.jse.2016.06.006
167. Vitali M, Cusumano A, Pedretti A, et al. Employment of synthetic patch with augmentation of the long head of the biceps tendon in irreparable lesions of the rotator cuff: our technique applied to 60 patients. *Tech Hand Up Extrem Surg* 2015;19(1):32-9. doi: 10.1097/BTH.0000000000000072 [published Online First: 2015/01/20]
168. Lenart BA, Martens KA, Kearns KA, et al. Treatment of massive and recurrent rotator cuff tears augmented with a poly-l-lactide graft, a preliminary study. *Journal of Shoulder and Elbow Surgery* 2015;24(6):915-21. doi: http://dx.doi.org/10.1016/j.jse.2014.09.044
169. Proctor C. Long-term successful arthroscopic repair of large and massive rotator cuff tears with a functional and degradable reinforcement device. *Journal of Shoulder and Elbow Surgery* 2014;23(10):1508-13. doi: http://dx.doi.org/10.1016/j.jse.2014.01.010
170. Cho CH, Lee SM, Lee YK, et al. Mini-open suture bridge repair with porcine dermal patch augmentation for massive rotator cuff tear: surgical technique and preliminary results. *Clin Orthop Surg* 2014;6(3):329-35. doi: 10.4055/cios.2014.6.3.329 [published Online First: 2014/09/02]
171. Venouziou A, Kokkalis ZS, DG. Human Dermal Allograft Interposition for the Reconstruction of Massive Irreparable Rotator Cuff Tears. *The American Journal of Orthopedics* 2013;42(2):63-70.
172. Petriccioli D, Bertone C, Marchi G, et al. Open repair of isolated traumatic subscapularis tendon tears with a synthetic soft tissue reinforcement. *Musculoskelet Surg* 2013;97 Suppl 1:63-8. doi: 10.1007/s12306-013-0260-5 [published Online First: 2013/04/17]
173. Mori D. Arthroscopic repair of irreparabale large to massive rotator cuff tears with low grade fatty degeneration of the infraspinatus: Patch autograft procedure versus partial repair procedure. *Arthroscopy - Journal*

of Arthroscopic and Related Surgery 2013;1):e152. doi: http://dx.doi.org/10.1016/j.arthro.2013.07.200
174. Gupta AK, Hug K, Boggess B, et al. Massive or 2-tendon rotator cuff tears in active patients with minimal glenohumeral arthritis: clinical and radiographic outcomes of reconstruction using dermal tissue matrix xenograft. *Am J Sports Med* 2013;41(4):872-9. doi: 10.1177/0363546512475204 [published Online First: 2013/02/21]
175. Marberry T. A Synthetic Reinforcement Patch in Repair of Challenging Two-Tendon Rotator Cuff Tears. *Shoulder & Elbow* 2013;5(1):24-29. doi: 10.1111/j.1758-5740.2012.00210.x
176. Gupta AK, Hug K, Berkoff DJ, et al. Dermal tissue allograft for the repair of massive irreparable rotator cuff tears. *Am J Sports Med* 2012;40(1):141-7. doi: 10.1177/0363546511422795 [published Online First: 2012/01/05]
177. Agrawal V. Healing rates for challenging rotator cuff tears utilizing an acellular human dermal reinforcement graft. *Int J Shoulder Surg* 2012;6(2):36-44. doi: 10.4103/0973-6042.96992 [published Online First: 2012/07/13]
178. Rotini R, Marinelli A, Guerra E, et al. Human dermal matrix scaffold augmentation for large and massive rotator cuff repairs: preliminary clinical and MRI results at 1-year follow-up. *Musculoskelet Surg* 2011;95 Suppl 1:S13-23. doi: 10.1007/s12306-011-0141-8 [published Online First: 2011/06/22]
179. Encalada-Diaz I, Cole BJ, Macgillivray JD, et al. Rotator cuff repair augmentation using a novel polycarbonate polyurethane patch: preliminary results at 12 months' follow-up. *J Shoulder Elbow Surg* 2011;20(5):788-94. doi: 10.1016/j.jse.2010.08.013 [published Online First: 2010/11/26]
180. Wong I, Burns J, Snyder S. Arthroscopic GraftJacket repair of rotator cuff tears. *J Shoulder Elbow Surg* 2010;19(2 Suppl):104-9. doi: 10.1016/j.jse.2009.12.017 [published Online First: 2010/03/10]
181. Phipatanakul W, Petersen S. Porcine Small Intestine Submucosa Xenograft Augmentation in Repair of Massive Rotator Cuff Tears. *Am J Orthop* 2009;38(11):572-75.
182. Bond JL, Dopirak RM, Higgins J, et al. Arthroscopic replacement of massive, irreparable rotator cuff tears using a GraftJacket allograft: technique and preliminary results. *Arthroscopy* 2008;24(4):403-09 e1. doi: 10.1016/j.arthro.2007.07.033 [published Online First: 2008/04/01]
183. Badhe S, Lawrence T, Smith F, et al. An assessment of porcine dermal xenograft as an augmentation graft in the treatment of extensive rotator cuff tears. *J Shoulder Elbow Surg* 2008;17(1 Suppl):35S-39S. doi: 10.1016/j.jse.2007.08.005 [published Online First: 2008/02/08]
184. Burkhead WZ, Schiffern SC, Krishnan SG. Use of Graft Jacket as an Augmentation for Massive Rotator Cuff Tears. *Seminars in Arthroplasty* 2007;18(1):11-18. doi: 10.1053/j.sart.2006.11.017
185. Audenaert E, Van Nuffel J, Schepens A, et al. Reconstruction of massive rotator cuff lesions with a synthetic interposition graft: a prospective study of 41 patients. *Knee Surg Sports Traumatol Arthrosc* 2006;14(4):360-4. doi: 10.1007/s00167-005-0689-7 [published Online First: 2005/10/28]

186. Malcarney HL, Bonar F, Murrell GA. Early inflammatory reaction after rotator cuff repair with a porcine small intestine submucosal implant: a report of 4 cases. *Am J Sports Med* 2005;33(6):907-11. doi: 10.1177/0363546504271500 [published Online First: 2005/04/14]
187. Sclamberg S, Tibone J, Itamura J, et al. Six-month magnetic resonance imaging follow-up of large and massive rotator cuff repairs reinforced with porcine small intestinal submucosa. *Journal of Shoulder and Elbow Surgery* 2004;13(5):538-41. doi: 10.1016/j.jse.2004.03.005
188. Metcalf M, Savoie F, Kellum B. Surgical technique for xenograft (SIS) augmentation of rotator-cuff repairs. *Operative Techniques in Orthopaedics* 2002;12(3):204-08. doi: 10.1053/otor.2002.36298
189. Hirooka A, Yoneda M, Wakaitani S, et al. Augmentation with a Gore-Tex patch for repair of large rotator cuff tears that cannot be sutured. *J Orthop Sci* 2002;7:451-56.
190. Hakimi O, Mouthuy PA, Zargar N, et al. A layered electrospun and woven surgical scaffold to enhance endogenous tendon repair. *Acta Biomater* 2015;26:124-35. doi: 10.1016/j.actbio.2015.08.007
191. Mouthuy P, Zargar N, Hakimi O, et al. Fabrication of continuous electrospun filaments with potential for use as medical fibres. *Biofabrication* 2015;7(2):025006. doi: 10.1088/1758-5090/7/2/025006 [published Online First: 2015/05/20]
192. Judge A, Murphy R, Maxwell R, et al. Temporal trends and geographical variation in the use of subacromial decompression and rotator cuff repair of the shoulder in England. *Bone Joint J* 2014;96-B(70) doi: 10.1302/0301-620X.96B1
193. Varghese M, Lamb J, Rambani R, et al. The use of shoulder scoring systems and outcome measures in the UK. *Ann R Coll Surg Engl* 2014;96(8):590-2. doi: 10.1308/003588414X14055925058157 [published Online First: 2014/10/29]
194. Dawson J, Hill G, Fitzpatrick R, et al. The benefits of using patient-based methods of assessment. *J Bone Joint Surg [Br]* 2001;83-B(6):877-82.
195. Dawson J, Hill G, Fitzpatrick R, et al. Comparison of clinical and patient-based measures to assess medium-term outcomes following shoulder surgery for disorders of the rotator cuff. *Arthritis Rheum* 2002;47(5):513-9. doi: 10.1002/art.10659 [published Online First: 2002/10/17]
196. De Carli A, Lanzetti RM, Monaco E, et al. The failure mode of two reabsorbable fixation systems: Swivelock with Fibertape versus Bio-Corkscrew with Fiberwire in bovine rotator cuff. *J Orthop Sci* 2012;17(6):789-95. doi: 10.1007/s00776-012-0275-z [published Online First: 2012/08/25]
197. Leger St-Jean B, Menard J, Hinse S, et al. Braided tape suture provides superior bone pull-through strength than wire suture in greater tuberosity of the humerus. *J Orthop* 2015;12(Suppl 1):S14-7. doi: 10.1016/j.jor.2015.01.025 [published Online First: 2016/01/01]
198. Liu RW, Lam PH, Shepherd HM, et al. Tape Versus Suture in Arthroscopic Rotator Cuff Repair: Biomechanical Analysis and Assessment of Failure Rates at 6 Months. *Orthop J Sports Med* 2017;5(4):2325967117701212. doi: 10.1177/2325967117701212 [published Online First: 2017/04/30]

199. Kim SJ, Kim SH, Moon HS, et al. Footprint Contact Area and Interface Pressure Comparison Between the Knotless and Knot-Tying Transosseous-Equivalent Technique for Rotator Cuff Repair. *Arthroscopy* 2016;32(1):7-12. doi: 10.1016/j.arthro.2015.07.004 [published Online First: 2015/09/19]
200. Kanazawa T, Gotoh M, Ohta K, et al. Novel characteristics of normal supraspinatus insertion in rats: an ultrastructural analysis using three-dimensional reconstruction using focused ion beam/scanning electron microscope tomography. *Muscles, Ligaments and Tendons Journal* 2014;4(2):182-87.
201. Benjamin M, Toumi H, Ralphs J, et al. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. *J Anat* 2006;208:471-90.
202. Carr AJ, Cooper CD, Campbell MK, et al. Clinical effectiveness and cost-effectiveness of open and arthroscopic rotator cuff repair [the UK Rotator Cuff Surgery (UKUFF) randomised trial]. *Health Technol Assess* 2015;19(80):1-218. doi: 10.3310/hta19800
203. Voigt C, Bosse C, Vosshenrich R, et al. Arthroscopic supraspinatus tendon repair with suture-bridging technique: functional outcome and magnetic resonance imaging. *Am J Sports Med* 2010;38(5):983-91. doi: 10.1177/0363546509359063
204. Charousset C, Bellaiche L, Kalra K, et al. Arthroscopic repair of full-thickness rotator cuff tears: is there tendon healing in patients aged 65 years or older? *Arthroscopy* 2010;26(3):302-9. doi: 10.1016/j.arthro.2009.08.027
205. Dwyer T, Razmjou H, Henry P, et al. Association between pre-operative magnetic resonance imaging and reparability of large and massive rotator cuff tears. *Knee Surg Sports Traumatol Arthrosc* 2015;23(2):415-22. doi: 10.1007/s00167-013-2745-z
206. Oh JH, Kim SH, Kang JY, et al. Effect of age on functional and structural outcome after rotator cuff repair. *Am J Sports Med* 2010;38(4):672-8. doi: 10.1177/0363546509352460
207. Collin P, Abdullah A, Kherad O, et al. Prospective evaluation of clinical and radiologic factors predicting return to activity within 6 months after arthroscopic rotator cuff repair. *J Shoulder Elbow Surg* 2015;24(3):439-45. doi: 10.1016/j.jse.2014.08.014
208. Cuff DJ, Pupello DR. Prospective evaluation of postoperative compliance and outcomes after rotator cuff repair in patients with and without workers' compensation claims. *J Shoulder Elbow Surg* 2012;21(12):1728-33. doi: 10.1016/j.jse.2012.03.002
209. Ahmad S, Haber M, Bokor D. The influence of intraoperative factors and postoperative rehabilitation compliance on the integrity of the rotator cuff after arthroscopic repair. *J Shoulder Elbow Surg* 2015;24(2):229-35. doi: 10.1016/j.jse.2014.06.050
210. Nho SJ, Shindle MK, Adler RS, et al. Prospective analysis of arthroscopic rotator cuff repair: subgroup analysis. *J Shoulder Elbow Surg* 2009;18(5):697-704. doi: 10.1016/j.jse.2008.11.018

211. Pennington WT, Gibbons DJ, Bartz BA, et al. Comparative analysis of single-row versus double-row repair of rotator cuff tears. *Arthroscopy* 2010;26(11):1419-26. doi: 10.1016/j.arthro.2010.03.013
212. Galatz LM, Gerstenfeld L, Heber-Katz E, et al. Tendon regeneration and scar formation: The concept of scarless healing. *J Orthop Res* 2015;33(6):823-31. doi: 10.1002/jor.22853 [published Online First: 2015/02/14]
213. Dawson J, Fitzpatrick R, Carr A. Questionnaire on the perceptions of patients about shoulder surgery. *Journal of Bone and Joint Surgery (British Volume)* 1996;78(4):593-600.
214. Roach K, Budiman-Mak E, Songiridej N, et al. Development of a shoulder pain and disability index. *Arthritis and Rheumatism* 1991;4(4):143-49.
215. Group" TE. EuroQol* - a new facility for the measurement of health-related quality of life. *Health Policy* 1990;16:199-208.
216. Cho NS, Rhee YG. The factors affecting the clinical outcome and integrity of arthroscopically repaired rotator cuff tears of the shoulder. *Clin Orthop Surg* 2009;1(2):96-104. doi: 10.4055/cios.2009.1.2.96
217. Mantovani M, Pellegrini A, Garofalo P, et al. A 3D finite element model for geometrical and mechanical comparison of different supraspinatus repair techniques. *J Shoulder Elbow Surg* 2016;25(4):557-63. doi: 10.1016/j.jse.2015.09.002 [published Online First: 2015/12/15]
218. Melis B, Nemoz C, Walch G. Muscle fatty infiltration in rotator cuff tears: descriptive analysis of 1688 cases. *Orthop Traumatol Surg Res* 2009;95(5):319-24. doi: 10.1016/j.otsr.2009.05.001 [published Online First: 2009/07/10]
219. Fuchs B, Weishaupt D, Zanetti M, et al. Fatty degeneration of the muscles of the rotator cuff: Assessment by computed tomography versus magnetic resonance imaging. *J Shoulder Elbow Surg* 1999;8(6):599-605.
220. Butt U, Rashid M, Temperley D, et al. Muscle regeneration following repair of the rotator cuff. *Bone Joint J* 2016;98-B(10):1389-94. doi: 10.1302/0301-620X.98B10
221. Carr AJ, Murphy R, Dakin SG, et al. Platelet-Rich Plasma Injection With Arthroscopic Acromioplasty for Chronic Rotator Cuff Tendinopathy: A Randomized Controlled Trial. *Am J Sports Med* 2015;43(12):2891-7. doi: 10.1177/0363546515608485 [published Online First: 2015/10/27]
222. Zimmer. Zimmer® Collagen Repair Patch http://www.zimmer.co.uk/content/dam/zimmer-web/documents/en-GB/pdf/medical-professionals/shoulder/Zimmer-Collagen-Repair-Patch-Brochure-97-4100-002-00-Rev-1-12-2006.pdf2006 [accessed 09/01/2018 2018.
223. NV. WMG. GRAFTJACKET™ Regenerative Tissue Matrix - "How is it processed?" [Web page]. http://www.wright.com/healthcare-professionals/graftjacket2017 [accessed 09/01/2018 2018.
224. Xu H WH, Zuo W, Sun W, Owens RT, Harper JR, Ayares DL, and McQuillan DJ. A Porcine-Derived Acellular Dermal Scaffold That Supports Soft Tissue Regeneration: Removal of Terminal Galactose-a-(1,3)-Galactose and Retention of Matrix Structure. *Tissue Eng Part A* 2009;15(7):1807-19.

225. Smith RDJ, Zargar N, Brown CP, et al. Characterizing the macro and micro mechanical properties of scaffolds for rotator cuff repair. *J Shoulder Elbow Surg* 2017;26(11):2038-46. doi: 10.1016/j.jse.2017.06.035 [published Online First: 2017/09/04]
226. McQuillan D, Harper J. A Novel Regenerative Tissue Matrix (RTM) Technology for Connective Tissue Reconstruction. *Wounds* 2007;19(6):163-68.
227. Xu H, Sandor M, Qi S, et al. Implantation of a porcine acellular dermal graft in a primate model of rotator cuff repair. *J Shoulder Elbow Surg* 2012;21(5):580-8. doi: 10.1016/j.jse.2011.03.014 [published Online First: 2011/07/05]
228. O'Brien JA, Ignotz R, Montilla R, et al. Long-term histologic and mechanical results of a Permacol abdominal wall explant. *Hernia* 2011;15(2):211-5. doi: 10.1007/s10029-010-0628-5 [published Online First: 2010/01/22]
229. Murphy R, Dean B, Wheway K, et al. A Novel Minimally Invasive Ultrasound-Guided Technique to Biopsy Supraspinatus Tendon. *Operative Techniques in Orthopaedics* 2013;23(2):56-62. doi: 10.1053/j.oto.2013.05.003
230. Uhlén M, Björling E, Agaton C, et al. A Human Protein Atlas for Normal and Cancer Tissues Based on Antibody Proteomics. *Mol Cell Proteomics* 2005;4(12):1920-32. doi: 10.1074/
231. Dakin SG, Buckley CD, Al-Mossawi MH, et al. Persistent stromal fibroblast activation is present in chronic tendinopathy. *Arthritis Res Ther* 2017;19(1):16. doi: 10.1186/s13075-016-1218-4
232. Barber F, Herbert M, Boothby M. Ultimate tensile failure loads of a human dermal allograft rotator cuff augmentation. *Arthroscopy* 2008;24(1):20-4. doi: 10.1016/j.arthro.2007.07.013 [published Online First: 2008/01/10]
233. Omae H, Steinmann SP, Zhao C, et al. Biomechanical effect of rotator cuff augmentation with an acellular dermal matrix graft: a cadaver study. *Clin Biomech (Bristol, Avon)* 2012;27(8):789-92. doi: 10.1016/j.clinbiomech.2012.05.001 [published Online First: 2012/05/23]
234. Chaudhury S, Holland C, Thompson MS, et al. Tensile and shear mechanical properties of rotator cuff repair patches. *J Shoulder Elbow Surg* 2012;21(9):1168-76. doi: 10.1016/j.jse.2011.08.045 [published Online First: 2011/11/15]
235. Smith RDJ, Carr A, Dakin SG, et al. The response of tenocytes to commercial scaffolds used for rotator cuff repair. *European Cells and Materials* 2016;31:107-18. doi: 10.22203/eCM.v031a08
236. Fini M, Torricelli P, Giavaresi G, et al. In vitro study comparing two collageneous membranes in view of their clinical application for rotator cuff tendon regeneration. *J Orthop Res* 2007;25(1):98-107. doi: 10.1002/jor.20295 [published Online First: 2006/10/21]
237. Derwin KA BA, Spragg RK, Leigh DR, and Iannotti JP. Commercial Extracellular Matrix Scaffolds for Rotator Cuff Tendon Repair. *Journal of Bone & Joint Surgery* 2006;88A(12):2665-72.
238. Snyder SJ, Arnoczky SP, Bond JL, et al. Histologic evaluation of a biopsy specimen obtained 3 months after rotator cuff augmentation with

GraftJacket Matrix. *Arthroscopy* 2009;25(3):329-33. doi: 10.1016/j.arthro.2008.05.023 [published Online First: 2009/02/28]
239. Soler J, Gidwani S, Curtis M. Early complications from the use of porcine dermal collagen implants (Permacol) as bridging constructs in the repair of massive rotator cuff tears A report of 4 cases. *Acta Orthop Belg* 2007;73(4):432-36.
240. Adams J, Zobitz M, Reach J, et al. Rotator cuff repair using an acellular dermal matrix graft: an in vivo study in a canine model. *Arthroscopy* 2006;22(7):700-9. doi: 10.1016/j.arthro.2006.03.016 [published Online First: 2006/07/18]
241. Kokkalis ZT, Mavrogenis AF, Scarlat M, et al. Human dermal allograft for massive rotator cuff tears. *Orthopedics* 2014;37(12):e1108-16. doi: 10.3928/01477447-20141124-59 [published Online First: 2014/12/02]
242. Ulery BD, Nair LS, Laurencin CT. Biomedical Applications of Biodegradable Polymers. *J Polym Sci B Polym Phys* 2011;49(12):832-64. doi: 10.1002/polb.22259 [published Online First: 2011/07/20]
243. Galloway MT, Lalley AL, Shearn JT. The role of mechanical loading in tendon development, maintenance, injury, and repair. *J Bone Joint Surg Am* 2013;95(17):1620-8. doi: 10.2106/JBJS.L.01004 [published Online First: 2013/09/06]
244. Group" WM. GRAFTJACKET™ TOTAL REGENERATIVE TISSUE MATRIX http://www.wright.com/healthcare-professionals/graftjacket2017 [Available from: http://www.wright.com/healthcare-professionals/graftjacket accessed 16/02/2018 2018.
245. Anderson J, KM. M. Biomaterial biocompatibility and the macrophage. *Biomaterials* 1984;5:5-10.
246. Anderson J. Inflammtory response to implants. *ASAIO transactions* 1988;34(2):101-07.
247. Anderson J, Rodriguez A, Chang D. Foreign body reaction to biomaterials. *Semin Immunol* 2008;20(2):86-100.
248. Zhang X, Bogdanowicz D, Erisken C, et al. Biomimetic scaffold design for functional and integrative tendon repair. *J Shoulder Elbow Surg* 2012;21(2):266-77. doi: 10.1016/j.jse.2011.11.016 [published Online First: 2012/01/17]
249. Morris GE, Bridge JC, Eltboli OM, et al. Human airway smooth muscle maintain in situ cell orientation and phenotype when cultured on aligned electrospun scaffolds. *Am J Physiol Lung Cell Mol Physiol* 2014;307(1):L38-47. doi: 10.1152/ajplung.00318.2013
250. Alvarez Perez M, Guarino V, Cirillo V, et al. In vitro mineralization and bone osteogenesis in poly(epsilon-caprolactone)/gelatin nanofibers. *J Biomed Mater Res A* 2012;100(11):3008-19. doi: 10.1002/jbm.a.34233
251. Leong NL, Kabir N, Arshi A, et al. Evaluation of polycaprolactone scaffold with basic fibroblast growth factor and fibroblasts in an athymic rat model for anterior cruciate ligament reconstruction. *Tissue Eng Part A* 2015;21(11-12):1859-68. doi: 10.1089/ten.TEA.2014.0366
252. Rowland DC, Aquilina T, Klein A, et al. A comparative evaluation of the effect of polymer chemistry and fiber orientation on mesenchymal stem

cell differentiation. *J Biomed Mater Res A* 2016;104(11):2843-53. doi: 10.1002/jbm.a.35829
253. Khan M, Dudhia J, David F, et al. Bone marrow mesenchymal stem cells do not enhance intra-synovial tendon healing despite engraftment and homing to niches with the synovium. *In Press* 2018
254. Depres-Tremblay G, Chevrier A, Snow M, et al. Rotator cuff repair: a review of surgical techniques, animal models, and new technologies under development. *J Shoulder Elbow Surg* 2016;25(12):2078-85. doi: 10.1016/j.jse.2016.06.009 [published Online First: 2016/08/25]
255. Hakimi O, Murphy R, Stachewicz U, et al. An electrospun polydioxanone patch for the localisation of biological therapies during tendon repair. *European Cells and Materials* 2012;24:344-57.
256. Wikipaedia. Twill Weave https://en.wikipedia.org/wiki/Twill: Wikipaedia; 2017 [accessed 14/10/2018 2018.
257. Manning CN, Schwartz AG, Liu W, et al. Controlled delivery of mesenchymal stem cells and growth factors using a nanofiber scaffold for tendon repair. *Acta Biomater* 2013;9(6):6905-14. doi: 10.1016/j.actbio.2013.02.008
258. Oryan A, Moshiri A, Meimandi Parizi A, et al. A long-term in vivo investigation on the effects of xenogenous based, electrospun, collagen implants on the healing of experimentally-induced large tendon defects. *J Musculoskelet Neuronal Interact* 2013;13(3):353-67.
259. Zhao S, Zhao J, Dong S, et al. Biological augmentation of rotator cuff repair using bFGF-loaded electrospun poly(lactide-co-glycolide) fibrous membranes. *Int J Nanomedicine* 2014;9:2373-85. doi: 10.2147/IJN.S59536
260. Kalfa D, Bel A, Chen-Tournoux A, et al. A polydioxanone electrospun valved patch to replace the right ventricular outflow tract in a growing lamb model. *Biomaterials* 2010;31(14):4056-63. doi: http://dx.doi.org/10.1016/j.biomaterials.2010.01.135
261. Pontailler M, Illangakoon E, Williams GR, et al. Polymer-Based Reconstruction of the Inferior Vena Cava in Rat: Stem Cells or RGD Peptide? *Tissue Eng Part A* 2015;21(9-10):1552-64. doi: 10.1089/ten.TEA.2014.0254 [published Online First: 2015/01/23]
262. Bhaskar P, Bosworth LA, Wong R, et al. Cell response to sterilized electrospun poly(varepsilon-caprolactone) scaffolds to aid tendon regeneration in vivo. *J Biomed Mater Res A* 2017;105(2):389-97. doi: 10.1002/jbm.a.35911
263. Yin Z, Chen X, Chen JL, et al. The regulation of tendon stem cell differentiation by the alignment of nanofibers. *Biomaterials* 2010;31(8):2163-75. doi: 10.1016/j.biomaterials.2009.11.083
264. Buschmann J, Meier-Burgisser G, Bonavoglia E, et al. Cellular response of healing tissue to DegraPol tube implantation in rabbit Achilles tendon rupture repair: an in vivo histomorphometric study. *J Tissue Eng Regen Med* 2013;7(5):413-20. doi: 10.1002/term.538
265. Yin Z, Chen X, Song HX, et al. Electrospun scaffolds for multiple tissues regeneration in vivo through topography dependent induction of lineage

specific differentiation. *Biomaterials* 2015;44:173-85. doi: 10.1016/j.biomaterials.2014.12.027
266. El-Fiqi A, Kim JH, Kim HW. Osteoinductive fibrous scaffolds of biopolymer/mesoporous bioactive glass nanocarriers with excellent bioactivity and long-term delivery of osteogenic drug. *ACS Appl Mater Interfaces* 2015;7(2):1140-52. doi: 10.1021/am5077759
267. Pan Y, Zhou X, Wei Y, et al. Small-diameter hybrid vascular grafts composed of polycaprolactone and polydioxanone fibers. *Sci Rep* 2017;7(1):3615. doi: 10.1038/s41598-017-03851-1 [published Online First: 2017/06/18]
268. Abdelshahed M, Mahure SA, Kaplan DJ, et al. Arthroscopic Rotator Cuff Repair: Double-Row Transosseous Equivalent Suture Bridge Technique. *Arthrosc Tech* 2016;5(6):e1297-e304. doi: 10.1016/j.eats.2016.07.022 [published Online First: 2017/02/06]
269. Park MC, McGarry MH, Gunzenhauser RC, et al. Does transosseous-equivalent rotator cuff repair biomechanically provide a "self-reinforcement" effect compared with single-row repair? *J Shoulder Elbow Surg* 2014;23(12):1813-21. doi: 10.1016/j.jse.2014.03.008 [published Online First: 2014/06/09]
270. Miller T, Feinblatt J, Craw J, et al. Evaluation of High-Strength Orthopedic Sutures: A Head-to-Head Comparison. *Orthopaedics* 2010;33(9):674.
271. Halder A, Zobitz M, Schultz F, et al. Mechanical properties of the posterior rotator cuff. *Clinical Biomechanics* 2000;15:456-62.
272. Ying ZM, Lin T, Yan SG. Arthroscopic single-row versus double-row technique for repairing rotator cuff tears: a systematic review and meta-analysis. *Orthop Surg* 2014;6(4):300-12. doi: 10.1111/os.12139 [published Online First: 2014/11/29]
273. Cummins CA, Murrell GA. Mode of failure for rotator cuff repair with suture anchors identified at revision surgery. *J Shoulder Elbow Surg* 2003;12(2):128-33. doi: 10.1067/mse.2003.21 [published Online First: 2003/04/18]
274. Cho NS, Yi JW, Lee BG, et al. Retear patterns after arthroscopic rotator cuff repair: single-row versus suture bridge technique. *Am J Sports Med* 2010;38(4):664-71. doi: 10.1177/0363546509350081 [published Online First: 2009/12/31]
275. Williams JF, Patel SS, Baker DK, et al. Abrasiveness of high-strength sutures used in rotator cuff surgery: are they all the same? *J Shoulder Elbow Surg* 2016;25(1):142-8. doi: 10.1016/j.jse.2015.07.018 [published Online First: 2015/10/01]
276. Levine H. Stitch in Time: 18 Fascinating Facts About the History of Sutures https://www.jnj.com/our-heritage/history-of-sutures-ethicon: Johnson & Johnson; 2016 [accessed 20/02/2018 2018.
277. Laufer N, Merino M, Trietsch H, et al. Macroscopic and histologic tissue reaction to polydioxanone, a new, synthetic, monofilament microsuture. *J Reprod Med* 1984;29(5):307-10.
278. Inui A, Kokubu T, Mifune Y, et al. Regeneration of rotator cuff tear using electrospun poly(d,l-Lactide-Co-Glycolide) scaffolds in a rabbit model.

Arthroscopy 2012;28(12):1790-9. doi: 10.1016/j.arthro.2012.05.887 [published Online First: 2012/10/13]
279. Iannotti JP, Deutsch A, Green A, et al. Time to failure after rotator cuff repair: a prospective imaging study. *J Bone Joint Surg Am* 2013;95(11):965-71. doi: 10.2106/JBJS.L.00708 [published Online First: 2013/06/20]
280. Evrova O, Houska J, Welti M, et al. Bioactive, Elastic, and Biodegradable Emulsion Electrospun DegraPol Tube Delivering PDGF-BB for Tendon Rupture Repair. *Macromol Biosci* 2016;16(7):1048-63. doi: 10.1002/mabi.201500455 [published Online First: 2016/04/14]
281. Liu S, Chen H, Wu T, et al. Macrophage infiltration of electrospun polyester fibers. *Biomater Sci* 2017;5(8):1579-87. doi: 10.1039/c6bm00958a [published Online First: 2017/02/23]
282. Zhao S, Dong S, Huangfu X, et al. Biological augmentation of rotator cuff repair using bFGF-loaded electrospun poly(lactide-co-glycolide) fibrous membranes. *International journal of nanomedicine* 2014;9:2373-85. doi: http://dx.doi.org/10.2147/IJN.S59536
283. McKeon-Fischer KD, Rossmeisl JH, Whittington AR, et al. In vivo skeletal muscle biocompatibility of composite, coaxial electrospun, and microfibrous scaffolds. *Tissue Eng Part A* 2014;20(13-14):1961-70. doi: 10.1089/ten.TEA.2013.0283 [published Online First: 2014/01/30]
284. Wang K, Zheng W, Pan Y, et al. Three-Layered PCL Grafts Promoted Vascular Regeneration in a Rabbit Carotid Artery Model. *Macromol Biosci* 2016;16(4):608-18. doi: 10.1002/mabi.201500355 [published Online First: 2016/01/13]
285. Seo YK, Kim JH, Eo SR. Co-effect of silk and amniotic membrane for tendon repair. *J Biomater Sci Polym Ed* 2016;27(12):1232-47. doi: 10.1080/09205063.2016.1188349 [published Online First: 2016/05/18]
286. Gigliobianco G, Chong CK, MacNeil S. Simple surface coating of electrospun poly-L-lactic acid scaffolds to induce angiogenesis. *J Biomater Appl* 2015;30(1):50-60. doi: 10.1177/0885328215569891 [published Online First: 2015/02/06]
287. "Arthrex". Shoulder Home https://www.arthrex.com/shoulder/products2018 [Available from: https://www.arthrex.com/shoulder/products accessed 22/02/2018 2018.
288. Nephew" S. Shoulder Repair http://www.smith-nephew.com/uk/products/sports-medicine/shoulder-repair/2018 [Available from: http://www.smith-nephew.com/uk/products/sports-medicine/shoulder-repair/ accessed 22/02/2018 2018.
289. "Stryker". Implants http://sportsmedicine.stryker.com/SolutionHub/1/Shoulder-Solutions2018 [Available from: http://sportsmedicine.stryker.com/SolutionHub/1/Shoulder-Solutions accessed 22/02/2018 2018.
290. "Biomet". Shoulder http://www.biomet.co.uk/medhome-uk/sportsmedicine/shoulder2018 [Available from: http://www.biomet.co.uk/medhome-uk/sportsmedicine/shoulder accessed 22/02/2018 2018.

291. Paxton ES, Teefey SA, Dahiya N, et al. Clinical and radiographic outcomes of failed repairs of large or massive rotator cuff tears: minimum ten-year follow-up. *J Bone Joint Surg Am* 2013;95(7):627-32. doi: 10.2106/JBJS.L.00255 [published Online First: 2013/04/05]
292. Bedi A, Dines J, Warren RF, et al. Massive tears of the rotator cuff. *J Bone Joint Surg Am* 2010;92(9):1894-908. doi: 10.2106/JBJS.I.01531 [published Online First: 2010/08/06]
293. Walter SG, Stadler T, Thomas TS, et al. Advanced Rotator Cuff Tear Score (ARoCuS): a multi-scaled tool for the classification and description of rotator cuff tears. *Musculoskelet Surg* 2018 doi: 10.1007/s12306-018-0535-y [published Online First: 2018/03/04]
294. Snyder S, Bond J. Technique for Arthroscopic Replacement of Severely Damaged Rotator Cuff Using "GraftJacket" Allograft. *Operative Techniques in Sports Medicine* 2007;15(2):86-94. doi: 10.1053/j.otsm.2006.07.006
295. Papalia R, Franceschi F, Zampogna B, et al. Augmentation techniques for rotator cuff repair. *British Medical Bulletin* 2013;105(1):107-38. doi: http://dx.doi.org/10.1093/bmb/lds029
296. Teefey S, Rubin D, Middleton W, et al. Detection and Quantification of Rotator Cuff Tears. *JBJS Am* 2004;86A(4):708-16.
297. Dudley L, Gamble C, Preston J, et al. What Difference Does Patient and Public Involvement Make and What Are Its Pathways to Impact? Qualitative Study of Patients and Researchers from a Cohort of Randomised Clinical Trials. *PLoS One* 2015;10(6):e0128817. doi: 10.1371/journal.pone.0128817 [published Online First: 2015/06/09]
298. Canada" RCoPaSo. CanMEDS: Better standards, better physicians, better care http://www.royalcollege.ca/rcsite/canmeds/canmeds-framework-e: Royal College of Physicians and Surgeons of Canada; 2011 [accessed 04/03/2018 2018.
299. Khoury V, Cardinal E, Brassard P. Atrophy and fatty infiltration of the supraspinatus muscle: sonography versus MRI. *AJR Am J Roentgenol* 2008;190(4):1105-11. doi: 10.2214/AJR.07.2835 [published Online First: 2008/03/22]
300. Jeong JY, Chung PK, Lee SM, et al. Supraspinatus muscle occupation ratio predicts rotator cuff reparability. *J Shoulder Elbow Surg* 2017;26(6):960-66. doi: 10.1016/j.jse.2016.11.001 [published Online First: 2017/02/06]
301. Tae SK, Oh JH, Kim SH, et al. Evaluation of fatty degeneration of the supraspinatus muscle using a new measuring tool and its correlation between multidetector computed tomography and magnetic resonance imaging. *Am J Sports Med* 2011;39(3):599-606. doi: 10.1177/0363546510384791 [published Online First: 2010/12/15]
302. Changa CY, Wanga SF, Chioua HJ, et al. Comparison of shoulder ultrasound and MR imaging in diagnosing full-thickness rotator cuff tears. *Clin Imag* 2002;26:50-54.
303. Pilling D, Fan T, Huang D, et al. Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS One* 2009;4(10):e7475. doi: 10.1371/journal.pone.0007475 [published Online First: 2009/10/17]

304. Lebaschi A, Deng XH, Zong J, et al. Animal models for rotator cuff repair. *Ann N Y Acad Sci* 2016;1383(1):43-57. doi: 10.1111/nyas.13203 [published Online First: 2016/10/11]
305. Research. NCftRRRoAi. The 3Rs https://www.nc3rs.org.uk/the-3rs2018 [accessed 17/03/2018 2018.

Appendices

Appendix 1: Reprint of Oxford Shoulder Score (OSS).

Shoulder Surgery Questionnaire – Before / after your operation

PROBLEMS WITH YOUR SHOULDER

Tick (✓) one box for every question.

1. During the past 4 weeks...

How would you describe the **worst** pain you had from your shoulder?

None	Mild	Moderate	Severe	Unbearable
☐	☐	☐	☐	☐

2. During the past 4 weeks...

Have you had any trouble dressing yourself because of your shoulder?

No trouble at all	A little bit of trouble	Moderate trouble	Extreme difficulty	Impossible to do
☐	☐	☐	☐	☐

3. During the past 4 weeks...

Have you had any trouble getting in and out of a car or using public transport because of your shoulder?

No trouble at all	A little bit of trouble	Moderate trouble	Extreme difficulty	Impossible to do
☐	☐	☐	☐	☐

4. During the past 4 weeks...

Have you been able to use a knife and fork - at the same time?

Yes, easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, impossible
☐	☐	☐	☐	☐

5. During the past 4 weeks...

Could you do the household shopping on your own?

Yes, easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, impossible
☐	☐	☐	☐	☐

6. During the past 4 weeks...

Could you carry a tray containing a plate of food across a room?

Yes, easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, impossible
☐	☐	☐	☐	☐

© Isis Innovation Limited, 1998. All rights reserved. Oxford Shoulder Score – English for the United Kingdom

7. During the past 4 weeks...

Could you brush/comb your hair with the affected arm?

Yes, easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, impossible
☐	☐	☐	☐	☐

8. During the past 4 weeks...

How would you describe the pain you usually had from your shoulder?

None	Very mild	Mild	Moderate	Severe
☐	☐	☐	☐	☐

9. During the past 4 weeks...

Could you hang your clothes up in a wardrobe, using the affected arm?

Yes, easily	With little difficulty	With moderate difficulty	With great difficulty	No, impossible
☐	☐	☐	☐	☐

10. During the past 4 weeks...

Have you been able to wash and dry yourself under both arms?

Yes, easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, impossible
☐	☐	☐	☐	☐

11. During the past 4 weeks...

How much has pain from your shoulder interfered with your usual work (including housework)?

Not at all	A little bit	Moderately	Greatly	Totally
☐	☐	☐	☐	☐

12. During the past 4 weeks...

Have you been troubled by pain from your shoulder in bed at night?

No nights	Only 1 or 2 nights	Some nights	Most nights	Every night
☐	☐	☐	☐	☐

Finally, please check back that you have answered each question.
Thank you very much.

© Isis Innovation Limited, 1998. All rights reserved. Oxford Shoulder Score – English for the United Kingdom