



## **Novel applications of biomaterials in the management of parastomal hernia and anal fistula**

Hammond, Toby Mark

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# **Novel Applications of Biomaterials in the Management of Parastomal Hernia and Anal Fistula**

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**By Toby Mark Hammond**

A thesis submitted for the higher degree of  
Doctorate in Medical Research (MD Res)

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## **Declaration of my contribution**

I, Toby Mark Hammond, confirm that all studies presented within this thesis have been my own work. The thesis was registered in January 2004 and submitted in April 2009. Assistance was kindly given in the preparation and interpretation of histological specimens. Anal physiology investigations and anal endosonography were performed and interpreted by colleagues within the GI Physiology Unit at The Royal London Hospital. All surgery on patients at both The Royal London and Homerton University Hospital was performed by Consultant Surgeons working at these institutions. I attended all surgical procedures on all patients where possible. I confirm that where information has been derived from other sources, this has been indicated in the thesis.

## Abstract

The aim of this thesis was to explore novel applications for both traditional and contemporary biomaterials in the management of parastomal hernia and anal fistula.

Parastomal hernias can be prevented or repaired using synthetic mesh; however, reported complications include infection, fibrosis and potential bowel erosion. The prophylactic role of a cross-linked collagen implant was assessed in terms of safety, feasibility and potential efficacy. Additionally, the human host response to this implant was evaluated. There were no complications related to infection or the implant's proximity to the bowel. The implant had excellent biocompatibility and resistance to degradation in most patients, and although fibrovascular in-growth and ECM deposition were limited, it seems to have excellent potential for soft tissue reinforcement and, more specifically, prevention of parastomal hernias.

Anal fistulas are in the main successfully treated by surgical fistulotomy, however damage to the anal sphincter complex and subsequent incontinence have led to the development of other techniques which aim to either lessen or avoid such disturbance. One strategy involves the traditional cutting seton, and a modification of this technique, the 'snug' silastic seton was assessed. In the short-medium term, this modification was demonstrated to be an effective addition to the fistula surgeon's armamentarium, although minor incontinence remained a concern. Other approaches employing contemporary biomaterials, fibrin glue and porcine intestinal submucosa, are aimed at tissue repair, rather than minimizing destruction. Their success rates however are highly variable. A pilot study aiming to assess the safety and potential efficacy of an

alternative biomaterial, cross-linked collagen in two different physical formats, was presented. In the short-medium term, both formats were shown to be safe, and equally effective. The results justify continued research into the use of biologically derived materials to heal anal fistulas.

In conclusion, although disparate pathologies were addressed, both they and the thesis are unified by demonstrating that an understanding of the specific disease pathology, wound healing, and the host response to materials (synthetic and biological) are central to their successful management.

## Acknowledgements

I should like to thank the following, whose help and support ensured the work presented was possible:

Dr Joanne Chin-Aleong and her team in the Division of Cellular Pathology at The Royal London Hospital, for their assistance in the preparation of all histological specimens, and in particular, Dr Chin-Aleong's personal help in interpretation of all pathological data.

Mr Andrew Huang for his assistance in obtaining ethical approval, and Mrs Karyn Prosser for helping record and collate all clinical data related to the parastomal hernia work.

Professor Norman Williams and Mr John Frye, who performed all the surgery on the parastomal hernia study patients, and Mr Peter Lunniss who performed all the surgery for the fistula studies. I am very grateful to both Professor Williams and Mr Lunniss who provided unstinting advice throughout the project.

Mrs Theresa Porrett for her constant support, and assistance in recording and collating clinical data for both fistula studies.

Dr Mark Scott for performing and interpreting anal endosonography and physiology investigations. Additionally his help in statistical analysis was greatly appreciated.

My greatest appreciation however goes to Mr Peter Lunniss, who supervised the evolution and eventual completion of this thesis. His indefatigable good natured support, understanding and incisive advice on both a personal and professional level has provided me with a role model on which I base my current and future career. Thank you.

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# Chapter 1

Background to thesis



## 1.1 Background

### 1.1.1 History of biomaterials

Conventionally biomaterials have been defined as substances other than drugs or foods contained in therapeutic or diagnostic systems that are in contact with tissue or biological fluids<sup>1</sup>. Throughout history, materials have played an important role in the treatment of disease: historians have traced sutures back 32,000 years, and metals such as gold were used in dentistry over 2000 years ago<sup>2:3</sup>. However, most early medical implants were doomed to failure because important concepts relating to infection, materials and the host reaction to materials were not yet established. It is only since the advent of synthetic polymers at the end of the nineteenth century, that the use of biomaterials in health care has soared. For example, polymethylmethacrylate (PMMA) was used in dentistry in the 1930s and cellulose acetate was used in dialysis tubing in the 1940s. Dacron was used to make vascular grafts; polyether-urethanes were used in artificial hearts; PMMA and stainless steel were used in total hip replacements<sup>4</sup>. Currently, biomaterials as initially defined are used in almost every branch of medicine. They are used in many pharmaceutical preparations (such as coatings for tablets or capsules, or as components of transdermal patches), they play a central role in extracorporeal devices (from contact lens to kidney dialysers), and are used extensively throughout all the surgical specialities, including cardiovascular surgery (prosthetic valves, vascular grafts, pacemakers and stents), plastic and reconstructive surgery (breast augmentation or reconstruction), orthopaedics (joint prostheses and fracture fixation), neurosurgery (cochlear implants and

hydrocephalus shunts) and general surgery (sutures, staples, tissue adhesives and meshes for hernia repair)<sup>1</sup>.

An appreciation of the host response to these implanted synthetic materials is an important step to understanding the need to develop more biocompatible materials that will assist, rather than be the focus of, the normal physiological healing response.

## 1.1.2 The healing response

### 1.1.2.1 The normal healing response

This is a complex and dynamic process of restoring cellular structures and tissue layers. Tissue injury initially results in haemorrhage, and subsequent vasoconstriction, after which four distinct phases can be identified: haemostasis, inflammation, proliferation, and remodelling<sup>5</sup>.

#### 1.1.2.1.1 Haemostasis

Following vasoconstriction, platelets adhere to damaged endothelium and discharge adenosine diphosphate (ADP), promoting thrombocyte clumping, which dams the wound<sup>6</sup>. The inflammatory phase is initiated by the release of numerous cytokines by platelets. Alpha granules liberate platelet-derived growth factor (PDGF), platelet factor IV, and transforming growth factor beta (TGF- $\beta$ ), while vasoactive amines such as histamine and serotonin are released from dense bodies found in thrombocytes<sup>7</sup>. PDGF is chemotactic for fibroblasts and along with TGF- $\beta$  is a potent modulator of fibroblastic mitosis, leading to prolific collagen fibril construction in later phases<sup>8</sup>. Fibrinogen is

cleaved into fibrin and the framework for completion of the coagulation process is formed. Fibrin provides the structural support for cellular constituents of inflammation. This process starts immediately after the insult and may continue for a few days<sup>8;9</sup>.

#### 1.1.2.1.2 Inflammation

Within the first 6-8 hours, the next phase of healing commences, with polymorphonuclear leucocytes (PMNs) predominating<sup>6</sup>. TGF- $\beta$  facilitates PMN migration from surrounding blood vessels, from which they extrude themselves. These cells “cleanse” the wound via phagocytocytosis. The PMNs attain maximal numbers in 24-48 hours and commence their departure by 72 hours<sup>6;9</sup>. Other chemotactic agents released include: fibroblastic growth factor (FGF), transforming growth factors (TGF- $\beta$  and TGF- $\alpha$ ), PDGF, and plasma-activated complements C3a and C5a (anaphylactic toxins). These are sequestered by macrophages or interred within the scab or eschar<sup>10</sup>.

As the process continues, monocytes also exude from the vessels, differentiating into macrophages. These continue the cleansing process, and manufacture crucial growth factors (TGFs, cytokines and interleukin-1 (IL-1), tumor necrosis factor (TNF), and PDGF) during days 3-4<sup>11</sup>. The macrophages orchestrate the multiplication of endothelial cells and subsequent neovascularisation, the duplication of smooth muscle cells (myofibroblasts), and in conjunction with recruited fibroblasts create a suitable wound milieu for repair<sup>12</sup>.

#### 1.1.2.1.3 Granulation

This phase can be subdivided into fibroplasia, matrix deposition, angiogenesis and re-epithelialization, which constitute an overall and ongoing process lasting up to 4 weeks in the clean and uncontaminated wound<sup>5</sup>. On days 5-7, fibroblasts migrate into the wound, laying down neo-collagen of the subtypes I and III. Early in normal wound healing, type III collagen predominates but is later replaced by type I collagen. The wound is also suffused with glycosaminoglycans (including include heparan sulfate, hyaluronic acid, chondroitin sulfate, keratan sulfate, and proteoglycans – glycosaminoglycans covalently bonded to a protein core, contributing to matrix deposition) and fibronectin produced by fibroblasts<sup>13</sup>.

Angiogenesis is the product of parent vessel offshoots. The formation of new vasculature requires extracellular matrix and basement membrane degradation followed by migration, mitosis, and maturation of endothelial cells. Basic FGF and vascular endothelial growth factor are believed to modulate angiogenesis<sup>14</sup>.

Re-epithelization occurs with the migration of cells from the periphery of the wound and adnexal structures. This process commences with the spreading of cells within 24 hours. Division of peripheral cells occurs in hours 48-72, resulting in a thin epithelial cell layer, which bridges the wound. Epidermal growth factors are believed to play a key role in this aspect of wound healing<sup>9;11</sup>.

#### 1.1.2.1.4 Remodelling

After the third week, the wound undergoes constant alterations, known as remodelling, which can last for years after the initial injury occurred<sup>9</sup>. Collagen

is degraded and deposited in equilibrium, resulting in no change in the amount of collagen present in the wound. The collagen deposition in normal wound healing reaches a peak by the third week after the wound is created. Contraction of the wound is an ongoing process resulting in part from the proliferation of the specialized fibroblasts termed myofibroblasts, which resemble contractile smooth muscle cells<sup>9:12</sup>.

The culmination of these biological processes can result in the complete restoration of tissue architecture, however in most cases granulation tissue is remodelled into fibroblastic mediated scar tissue.

#### 1.1.2.1.5 Biological response to traditional biomaterials

Synthetic biomaterials tend to result in the formation and organisation of granulation tissue with subsequent fibrosis, often succeeded by the development of a fibrous capsule at the tissue/ material interface<sup>15</sup>.

The initial event upon implantation of the biomaterial is non-specific protein adsorption, known as the Vroman effect<sup>16</sup>. Many proteins adsorb to the surface in a range of conformations from native to denatured. However, non-specific protein adsorption never occurs in the normal physiological process of wound healing, and therefore may be an instigator in the response seen. A number of key inflammatory cells (monocytes, macrophages, leucocytes and platelets) adhere to the biomaterial surface, and as a result may lead to the up-regulation of various cytokines and subsequent pro-inflammatory processes<sup>3</sup>. Additionally, biomaterials are not generally phagocytosed, due to size disparity between the material and the attached cell, which can lead to 'frustrated phagocytosis'. These persistent physical and chemical

inflammatory stimuli lead to a chronic inflammatory response, characterised by the predominance of macrophages. This response essentially involves two processes: the extracellular release of various proteases in an attempt to degrade the material, and is specifically dependant on the size of the implant (e.g. a material in a phagocytosable form, powder or particulate, may provoke a degree of inflammatory response different from that of the same material in a non-phagocytosable form, such as a sheet); and the fusion of the frustrated macrophages to form multi-nucleated foreign body giant cells that often persist for the lifetime of the implant<sup>15;17</sup>. The end-stage of the foreign body reaction involves the walling off of the implant by an avascular, collagenous fibrous tissue that is typically 50-200 µm thick<sup>3</sup>.

Traditional biomaterials therefore initiate an unplanned stochastic biological response. Chronic inflammation, contracture of implants, and the formation of a surrounding fibrous capsule can lead to implant failure, chronic pain and the development of specific site complications. Thus, although traditional synthetic biomaterials have played a crucial role in the management of a variety of medical disorders, there is a substantial role for improvement, and this should be based on the knowledge of the biology of wound healing and inflammation, and the crucial role of the extracellular matrix in these mechanisms.

### **1.1.3 Tissue engineering and the extracellular matrix**

In recent years, the definition of biomaterials has been broadened to include materials composed of biologically derived components irrespective of their application<sup>4</sup>. An area where this has recently had an impact is in tissue

engineering and regenerative medicine, whereby materials composed of naturally occurring extracellular matrix (ECM) components are being studied for applications such as direct tissue repair, regeneration and specific de novo tissue or organ production.

The ECM is a vital dynamic and indispensable component of all tissues and organs and is the native scaffold for tissue and organ morphogenesis, maintenance, and reconstruction following injury<sup>18</sup>.

Up until the last two decades it was generally accepted that the ECM was simply an inert scaffold stabilizing the physical structure of tissues; a tissue component that interconnected (functionally important) tissues, hence the term connective tissue, the cement or glue between the elements that really mattered. However it is now accepted that the ECM is actually a dynamic 'virtual information highway': dynamic, as it is subject to constant renewal, serves a crucial architectural role during foetal development and tissue repair, and is interactive. Adjacent parenchymal cells deposit matrix molecules, which simultaneously provide cues that modulate the functional activity of these cells<sup>19;20</sup>.

The ECM is a complex mixture of structural and functional proteins arranged in a unique, tissue specific three-dimensional ultrastructure<sup>21</sup>. At this ultrastructural level, it is composed of two domains, the interstitial matrix and the basement membrane. The latter is a condensed matrix layer that is formed adjacent to epithelial cells, other covering cell sheets (e.g. mesothelium), muscle cells, and adipocytes. The main characteristic these two domains have in common is that a collagen scaffold defines their basic structure, although the collagens that make up the scaffold are quite different,

as are their three-dimensional architecture. Adhesive glycoproteins, including laminin and tenascin, and proteoglycans (via their side-chain glycosaminoglycans) adhere to the scaffold and interact with the cells in or adjacent to the matrix<sup>20</sup>. These proteins collectively serve many functions including the provision of structural support and tensile strength, and act as a reservoir for growth factors (such as fibroblast growth factor, vascular endothelial growth factor and epidermal growth factor) and cytokines that modulate such diverse host processes as angiogenesis, cell migration, cell proliferation and orientation, inflammation, immune responsiveness and wound healing<sup>18;22;23</sup>. The extracellular matrix is not static: it is remodelled constantly, which implies constant breakdown by proteases, notably the family of matrix metalloproteases<sup>20;24</sup>. Furthermore, the composition and structure of the ECM are a function of their location within tissues and organs, the age of the host, and the physiological requirements of the particular tissue<sup>18</sup>. The ECM interacts with surrounding cells by efficiently presenting various signalling factors, via matrix receptors (of which the integrins constitute the most important class), to attachment sites for cell surface receptors. The ECM also protects these factors from degradation and modulates their synthesis. In this manner, the ECM affects local concentrations and biologic activity of growth factors and cytokines<sup>21;25</sup>.

These intertwined structural and biological properties of the ECM have led to attempts to translate this interactive scaffold into a therapeutic use for tissue repair and reinforcement.



#### 1.1.4 The collagen scaffold

Collagen is the most abundant, ubiquitous and well-characterised protein within the ECM<sup>19</sup>. It is responsible for maintaining the structural integrity of organisms across both the animal and plant kingdoms<sup>26</sup>. More than 20 distinct types of collagen have been identified, although the most prevalent form found in mammalian tissues is type I collagen, of which allogenic and xenogeneic sources have been long recognised as effective biologic scaffolds for tissue repair with low antigenic potential<sup>18;25</sup>.

Type I collagen occurs throughout the body, except in cartilage. It is the principle collagen in dermis, fascia and tendons and is a major component of mature scar tissue. Type II collagen occurs in cartilage, the developing cornea and in the vitreous body of the eye<sup>20</sup>. Type III collagen is predominant within immature scar tissue and the wall of blood vessels, intestines and the urinary bladder, where non rigid structure is demanded for appropriate function. Type IV collagen is present within the basement membrane of all vascular structures and is an important ligand for endothelial cells<sup>25</sup>. Some collagens associate with fibril surfaces, such as subtype VII, which is the principal component anchoring fibrils of keratinocytes to the underlying basement membrane of the epidermis, and others such as subtype VI connect glycosaminoglycans to type I collagen, helping to maintain a gel-like consistency to the ECM<sup>18</sup>.

Collagens are mostly synthesized by proliferating cells within the ECM, such as fibroblasts, myofibroblasts, osteoblasts and chondrocytes. Some collagens are also synthesized by adjacent parenchymal or covering (epithelial, endothelial or mesothelial) cells. The production of specific collagen sub-types

is not only influenced by cell type, but also by the impact of both systemic and local factors on these cells, including the role of growth factors, inflammatory mediators and mechanotransduction on cell signalling and receptor mechanisms<sup>27</sup>. Alpha chains containing up to 1000 amino acids are converted, within the endoplasmic reticulum, into rod-shaped molecules of procollagen. In an early crucial step, with molecular oxygen as the substrate and vitamin C as the essential co-factor, proline and lysine are hydroxylated into hydroxyproline and hydroxylysine, which form interchain hydrogen bonds that stabilise the triple-stranded helix of procollagen<sup>28</sup>. Extracellularly, in tissues that have to resist shear, tensile or pressure forces (such as fascia, tendons, bone cartilage and skin) the procollagen terminal peptides are cleaved, by specific procollagen metalloproteases, and following the formation of strong covalent bonds between lysine and hydroxylysine residues, the collagen is arranged in parallel bundles of fibrils approximately 300nm in length and 1.5nm wide with a characteristic 67nm axial cross-striation<sup>20</sup>.

Senescent or damaged collagen fibres are degraded and replaced in a continuous controlled process of remodelling. Some matrix metalloproteases (MMPs) and serine proteases are perceived to specifically degrade collagen, and these are characterised as collagenases<sup>24;28</sup>. MMP activity is controlled at least at 3 levels: transcription, proteolytic activation, and inhibition of the active enzyme by tissue inhibitors of metalloproteases (TIMPs)<sup>24</sup>. Of the main collagenases, fibroblast collagenase (MMP-1) plays a distinctive role in eliminating defective procollagens during the formation of new collagen fibres, whereas neutrophil collagenases (MMP-8, MMP-9) are secreted during the inflammatory phase of wound healing<sup>28</sup>.

The diversity of collagens and their unique roles within the ECM exemplifies the benefits of employing these biomolecular components as tissue repair materials. Perhaps more importantly it indicates a potential role for utilising materials composed of specific collagen sub-types, which are therefore tailored both to the nature of repair required and the anatomical location of the defect.

### **1.1.5 Selected clinical applications of biomaterials**

The field of biomaterials has been essential to the development of surgery, allowing for the expansion of existing treatments and the creation of new techniques. Surgeons are uniquely positioned to contribute to the ongoing development and clinical application of biomaterials, but material selection must be based on an understanding of the materials available and their basic properties<sup>29</sup>. Two clinical conditions that have traditionally benefited from the therapeutic application of biomaterials are parastomal hernia and anal fistula. Current therapies in the management of these conditions have utilised synthetic materials and materials that use selected biomolecular components of the ECM but to date they have had limited success. The aim of this thesis was to identify novel applications of both traditional and contemporary biomaterials in the management of these conditions based on an understanding of the specific biochemical and mechanical properties required to optimise successful tissue repair.

## 1.2 Parastomal Hernia

### 1.2.1 Introduction

'Some degree of herniation around a stoma is so common that this complication may be regarded as inevitable'<sup>30</sup>

Goligher, 1984

A parastomal hernia is an incisional hernia related to an abdominal wall stoma<sup>31</sup>; that is, the protusion of any organ (or part thereof), other than the intended stoma, through an abdominal wall trephine created for the sole purpose of stoma formation. Parastomal herniation is considered to be an inevitable complication of stoma formation<sup>32</sup>, and their management is a common clinical dilemma, as once established they are notoriously difficult to treat<sup>33-35</sup>.

A stoma is a surgically created opening of the bowel or urinary tract to a body surface. Stomas are defined according to their purpose (defunctioning, usually temporary, loop stomas; and end or terminal, which are usually permanent) and the organ that they involve (e.g. colon, ileum, jejunum, ureter). Loop stomas are created to protect anastomoses or to divert luminal content from diseased segments of bowel (e.g. tumours or perianal fistulas). End stomas are usually created when the diseased segment of bowel cannot be salvaged. The thesis herein is concerned only with herniation following ileostomy and colostomy formation.

Parastomal hernias can cause a wide spectrum of complications ranging from mild to life threatening. These include poor cosmesis, psychological distress,

parastomal discomfort or pain, difficulty with appliance application (resulting in leakage of contents and possible skin irritation), and obstructive episodes, ranging from intermittent symptoms to incarceration, strangulation, and necrosis<sup>31;36-38</sup>. The contents of the hernia sac may include omentum, small bowel, stomach and colon, and these will determine the nature of the symptoms<sup>37;39;40</sup>.

### 1.2.2 Incidence

Reported incidence rates vary widely depending on the type of stoma, length of follow-up and the mode of detection<sup>33;35</sup>. Parastomal hernias are usually diagnosed clinically, but where uncertainty exists (or as part of a study methodology) radiological imaging, in particular computer tomography, has a proven role, and those studies that employed imaging as part of their follow-up protocol consistently reported higher herniation rates than those which used clinical examination alone<sup>41-43</sup>. Reported incidence rates for parastomal hernias range from 0 to 78%, increasing with the duration of follow-up<sup>33;35;41;42</sup>. Most will, however, develop within the first 12 months of formation, although the risk of herniation extends to the lifetime of the stoma<sup>44;45</sup>. In a recent review article, Carne *et al* detailed herniation rates for specific intestinal stoma types<sup>33</sup>, and these are summarised in Table 1.1. Interestingly, although loop stomas have the lowest rate of incidence, most likely on account of their predominantly temporary nature and therefore their comparatively shorter follow-up, they are considered more prone to herniation. This is because their construction requires a larger abdominal trephine compared with an end

stoma, which theoretically places them at greater risk of developing a parastomal hernia in the longer term<sup>44</sup>.

**Table 1.1.** Parastomal herniation rates for specific intestinal stoma types<sup>33</sup>

	Rate of herniation (%)	Length of follow up (months)
End colostomy	4.0 – 48.1	35 – 120
Loop colostomy	0 - 30.8	2 - 96
End ileostomy	1.8 – 28.3	31 – 110
Loop ileostomy	0 – 6.2	2 – 4

### 1.2.3 Aetiology

Parastomal hernia development is influenced by both patient and technical factors<sup>46</sup>.

#### 1.2.3.1 Patient factors

These are considered, acting either alone or in conjunction with each other, to weaken the edges of the abdominal wall trephine or increase the pressure under which they are subjected, thereby precipitating hernia formation. They are similar to those that are thought to influence other types of abdominal wall hernias, and traditionally include: obesity, malnutrition, raised intra-abdominal pressure (secondary to chronic coughing, straining at micturition or defaecation, ascites, or heavy-lifting), corticosteroid use, malignancy,

increasing age, post-operative wound sepsis, and previous abdominal surgery<sup>31;46-49</sup>. However, the aetiological basis of these precipitants is based on expert opinion rather than scientific fact<sup>3</sup>. More recently, it has been proposed that disturbances in collagen metabolism contribute to recurrent and incisional hernias (which by definition include parastomal hernias)<sup>50</sup> (Franz 2008). These include a pathological shift of the collagen ratio within the healed wound, from “mature” type I collagen to “immature” type III collagen, and the over expression of matrix metalloproteases (MMP), both of which may result in a loss of tensile strength and predispose to hernia formation<sup>50-53</sup>. The increased incidence of hernias in those with mutations of the collagen gene, Col3A1, associated with familial arterial aneurysm and Ehlers Danlos syndromes types III and IV, implies that polyfactorial mutations in the coding sequences of collagen genes may be partly responsible<sup>50</sup>. Tobacco smoking has also been implicated, as it is a potent activator of the proteases (including elastase and collagenase) and decreases anti-protease activity, and led to one group defining abdominal wall hernias as ‘metastatic emphysema’<sup>54</sup>.

### **1.2.3.2 Technical factors**

Technical factors that are traditionally thought to influence herniation include: size of the abdominal wall trephine, trephine location, stoma fixation to the abdominal wall fascia, closure of the lateral space and whether constructed electively or as an emergency<sup>33</sup>.

Various trephine sizes have been suggested, ranging from 1-2 fingerbreadths, to two-thirds the width of intestine intended for stoma formation, to a more precise 1.5 cm and 2 cm diameter ostomy site for colostomies and ileostomies

respectively<sup>55-57</sup>. It is difficult to determine any meaningful comparisons between these strategies as relevant studies are not controlled and numbers of participants and follow up are limited. One study, which retrospectively compared parastomal herniation to stoma size suggested that an aperture greater than 2cm is associated with an increased rate of hernia formation<sup>58</sup>. It has been calculated that the tangential force on the abdominal wall trephine is proportional to the radial force on the abdominal wall and the radius of the trephine<sup>59</sup>, meaning that obese patients (large abdominal wall radius) with large abdominal wall trephines (loop compared to end stomas, and colostomies compared to ileostomies) are at highest risk of herniation. At present, however, the best guide appears to be that one should create the smallest opening which allows the creation of a viable stoma without ischaemia<sup>33</sup>.

Current accepted operative technique involves creating a trephine through the rectus abdominis muscle, preferably at a pre-marked skin site. However, in 6 studies that specifically compared herniation rates between those stomas formed through the rectus abdominis muscle and those formed lateral to the muscle<sup>43-45;60-62</sup>, only one revealed any significant difference in parastomal herniation, in favour of the former technique<sup>61</sup>. There is no evidence to support the notion that fixation of the stoma to the abdominal wall fascia<sup>62</sup>, or that closure of the space lateral to the stoma reduces herniation<sup>33</sup>. Increased incidence of parastomal herniation in stomas created as an emergency has been proposed<sup>46</sup>, but this is not confirmed by the literature<sup>33</sup>.



#### 1.2.4 Assessment

Diagnosis is mostly by clinical examination<sup>35;46</sup>. A parastomal hernia should be considered present if there is any palpable defect or bulge adjacent to the stoma with the patient supine and legs elevated, or erect and coughing or straining<sup>35</sup>. In those patients whose symptoms are suggestive of a hernia, but in whom this cannot be clinically demonstrated, consideration should be given to radiological investigation<sup>43</sup>. To date, the preferred method of imaging is by computer tomography, which has been shown to significantly increase the diagnostic accuracy of parastomal hernia detection, as well as permitting pre-operative classification<sup>43;63-65</sup>.

#### 1.2.5 Classification

Parastomal hernias have been classified into 4 subtypes: subcutaneous, where the sac of the hernia lies in the subcutaneous tissues; interstitial, where the hernia sac lies within the abdominal wall layers; peristomal, with the bowel prolapsing through a circumferential hernia sac enclosing the stoma; and intrastomal, where in ileostomies, the hernia sac lies between the intestinal wall and everted intestinal layer<sup>47</sup>. However, there are no data attributing difference, in terms of symptoms or outcomes of repair, between the variously described subtypes, and as such the classification has not become widely used. Recently a new clinico-radiological classification system has been proposed which differentiates parastomal hernias according to the contents of the hernia sac, as well as the relationship between the hernia sac and the bowel forming the stoma<sup>42</sup>.

## 1.2.6 Management

‘A surgeon can do more for the community by operating on hernia cases and seeing his recurrence rate is low than he can by operating on cases of malignant disease.’

Sir Cecil Wakely, 1948

President, Royal College of Surgeons

A proportion of parastomal hernias can be managed conservatively, with or without the use of a stoma supporting device. However, up to 70% of patients will require surgical repair for treatment of their associated symptoms<sup>33;35</sup>. The surgical techniques include local tissue repair, stoma relocation, and mesh repair. The former two procedures have largely been superseded by mesh repair, which has become widely accepted as the operation of choice given its perceived lower hernia recurrence rate, although interestingly, the only study which clearly, albeit retrospectively, compares the 3 techniques showed no significant difference in recurrence rates between them<sup>66</sup>.

### 1.2.6.1 Non-mesh repair

Local tissue repair involves simple suture closure of the edges of the abdominal wall trephine, lateral to the stoma, to close the defect through which the hernia passed. Reported recurrence rates range from 46-100%<sup>33;35</sup>, and as such certain authors state that use of this technique cannot be justified, unless all other strategies are contraindicated<sup>35</sup>. Stoma relocation to

a new position on the abdominal wall has a lower hernia recurrence rate than local tissue repair<sup>67</sup>, but nonetheless still ranges from 24 – 86%<sup>35</sup>. Relocation can be achieved with or without formal laparotomy<sup>68</sup>, the advantage of the latter being improved recovery time, less post-operative pain and avoidance of another site for potential herniation<sup>69</sup>. Relocation to the other side of the abdominal wall is associated with lower recurrence than ipsilateral relocation<sup>69;70</sup>, but regardless of technical considerations, incisional hernia formation at the original stoma site is also a concern, with reported rates ranging from 8-52%<sup>67;69</sup>.

#### **1.2.6.2 Mesh repair**

The proven advantages of mesh repair for other forms of abdominal wall hernias fuelled the development of a similar strategy for parastomal hernias<sup>71;72</sup>. First described in 1977<sup>73</sup>, there are now over 70 reports in the literature on parastomal hernia mesh repair, with differing techniques described ranging from the ideal anatomical location to site the mesh (fascial onlay, preperitoneal or intraperitoneal)<sup>33;35</sup>, to mesh fixation and the type of mesh used (polytetrafluoroethylene, polypropylene, polyvinylidene, composite or biological)<sup>73-81</sup>, to the surgical approach (open or laparoscopic)<sup>81-87</sup> employed. The multitude of techniques and meshes used is testament to the fact that hernia recurrence rates are still high (overall recurrence for all types of mesh repair is reported as 7.8%<sup>33</sup>) and additionally highlights the unique challenges imposed on surgeons by the complications associated with mesh implantation in close proximity to bowel. The majority of studies tend toward retrospective case series, often employing small numbers of patients with a

limited duration of follow-up, therefore there are currently little data to support the use of one technique or mesh type over another. One retrospective study, compared surgical approach (transabdominal versus parastomal), mesh placement (onlay versus sublay), and mesh type (polypropylene versus polytetrafluoroethylene, PTFE), and despite an overall hernia recurrence of 63% (10 of 16 repairs), and wound infection rate of 11% (including 1 incidence of mesh erosion into bowel resulting in a colocutaneous fistula), none of the individual operative variants were deemed to be significantly associated with these outcomes<sup>88</sup>.

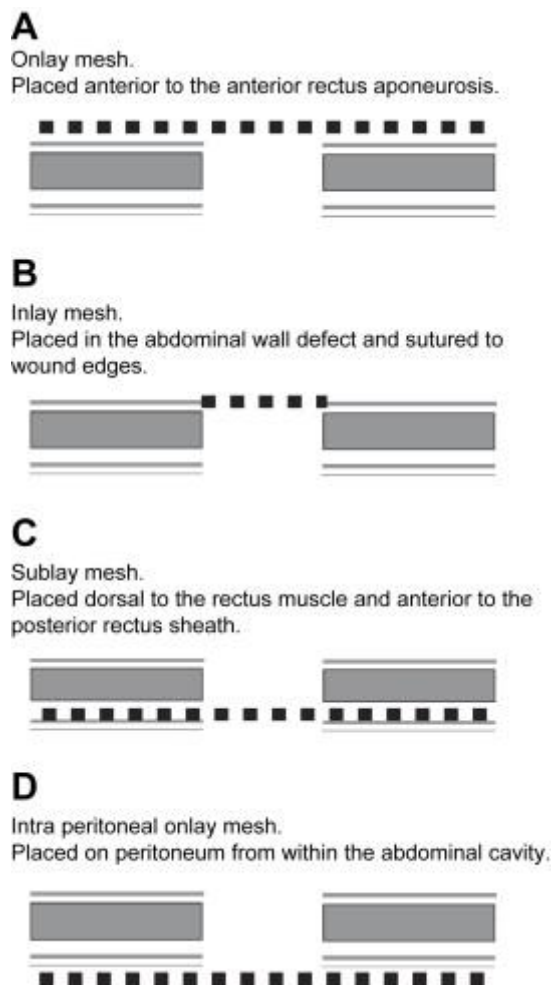
#### 1.2.6.2.1 Anatomical site

The fascial onlay technique involves siting the mesh on the anterior layer of the rectus sheath; the pre-peritoneal or sublay position is in between the rectus abdominis muscle and the posterior layer of the rectus sheath/peritoneum; and, intraperitoneal mesh placement involves attachment to the visceral surface of the peritoneum<sup>33;35</sup> (Figure 1.1).

##### 1.2.6.2.1.1 **Fascial onlay**

This was the first described parastomal hernia mesh repair technique<sup>73</sup>, and is still a commonly used approach<sup>80;86;89-93</sup>. It involves mobilization of the stoma at the mucocutaneous junction and suture repair of the fascial defect, followed by mesh placement. This technique requires the mesh to be securely anchored to the anterior rectus sheath, to avoid displacement secondary to raised intra abdominal pressures, and therefore requires extensive

mobilization of the surrounding tissue, increasing the risk of seroma formation and consequent mesh contamination<sup>35</sup>. Skin incision lateral to the stoma, aimed at lessening the risk of mesh contamination, has been described<sup>80;86;89</sup>, but has not been widely employed. Overall, the studies reporting on this technique are not randomised, and have a limited duration of follow-up (4 - 48 months). Rates of recurrence and mesh related complications range from 0 – 37.5% and 0 – 27.5% respectively (see Table 1.2). The 2 studies that employed biological mesh, in an attempt to reduce mesh related complications<sup>74;94</sup>, had similar rates of hernia recurrence to those studies which used synthetic mesh<sup>66;86</sup>, and although no specific mesh related complications were reported, the numbers involved are too small, and length of follow up too limited, to determine any benefit derived from their use.



**Figure 1.1** Mesh positions for ventral hernia repair. (A) Onlay mesh, placed anterior to the anterior rectus aponeurosis. (B) Inlay mesh, of historical interest only, placed in the abdominal wall defect and sutured to wound edges. (C) Sublay mesh, placed dorsal to the rectus muscle and anterior to the posterior rectus sheath. (D) Intra peritoneal onlay mesh (IPOM), placed on peritoneum from within the abdominal cavity. This figure is a reprint from reference 6 (reproduced with permission by Elsevier).

**Table 1.2** Published results for fascial onlay mesh repair of parastomal hernias.

Author	Year	No. of patients	Mesh	Follow-up (months)	Recurrence (%)	Other complications
Rosin & Bonardi <sup>73</sup>	1977	7	Polypropylene (Marlex)	4 – 48	0	0
Abdu <sup>95</sup>	1982	4	Polypropylene (Marlex)	48	0	1 wound infection
Bayer <i>et al</i> <sup>96</sup>	1986	7	Polypropylene (Marlex)	48	0	2 mesh infections
Tekkis <i>et al</i> <sup>80</sup>	1999	5	Polypropylene (lateral approach)	21	0	1 haematoma 1 stomal prolapse
Kald <i>et al</i> <sup>86</sup>	2001	3	Polypropylene (lateral approach)	12	1 (33%)	0
Amin <i>et al</i> <sup>89</sup>	2001	9	Polypropylene (lateral approach)	6	0	0
Venditti <i>et al</i> <sup>97</sup>	2001	8	Polypropylene	36	0	1 wound infection
Reiger <i>et al</i> <sup>66</sup>	2004	18	Synthetic - ?type	44	7 (34%)	7 wound infections 3 mesh infections 2 bowel fistulas
Kanellos <i>et al</i> <sup>87</sup>	2004	4	Polypropylene (lateral approach)	36	0	1 skin necrosis
Kish <i>et al</i> <sup>94</sup>	2005	3	Acellular dermal matrix (Alloderm)	12	1 (33%)	0
Aycock <i>et al</i> <sup>74</sup>	2007	8	Acellular dermal matrix (Alloderm)	9	3 (37.5%)	2 wound infections
Guzman-Valdivia <i>et al</i> <sup>85</sup>	2008	25	Light-weight polypropylene	12	2 (8%)	2 seromas 2 wound infections

### 1.2.6.2.1.2 Pre-peritoneal (sublay)

This has been proposed as the most advantageous technique for mesh repair of parastomal hernias<sup>35;98-100</sup>. Intra abdominal pressure will help secure rather than displace the mesh, as well as reduce the potential space for seroma accumulation, and the peritoneal layer keeps the amount of bowel to mesh contact to a minimum, thereby reducing the potential for mesh related complications<sup>35</sup>. The majority of published reports of this technique have described an open approach employing polypropylene mesh<sup>99;101-104</sup>, one of which was complicated by erosion of the mesh into an end colostomy<sup>101</sup>. One case report describes a laparoscopic approach (not dissimilar to the transabdominal pre-peritoneal (TAPP) inguinal hernia repair<sup>105;106</sup>), employing an expanded PTFE mesh, whereby a peritoneal flap was used to protect the bowel<sup>107</sup>, with no recurrence at 12 months. The pre-peritoneal technique is associated with a recurrence rate of 0 - 2% at up to 5 years follow up<sup>99;102;104</sup> (see Table 1.3).

**Table 1.3** Published results for pre-peritoneal (sublay) mesh repair of parastomal hernias

Author	Year	No. of patients	Mesh	Follow-up (months)	Recurrence (%)	Other complications
Kasperk <i>et al</i> <sup>99</sup>	2000	7	Light weight polypropylene	4 - 36	2	0
Egun <i>et al</i> <sup>102</sup>	2002	10	Polypropylene	54 (22 – 69)	0	<ul style="list-style-type: none"> <li>• 2 seromas</li> <li>• 2 wound infections</li> <li>• 1 mesh erosion → colostomy</li> <li>• 1 stoma infarction</li> </ul>
Longman & Thompson <sup>104</sup>	2005	10	Polypropylene	30 (2 – 40)	0	0



### **1.2.6.2.1.3 Intra-peritoneal**

Both open<sup>75;108-111</sup> and laparoscopic<sup>76;77;81;83;112-118</sup> approaches have been described for the intra-peritoneal placement of mesh, and their comparative merits and complications, in regards to incisional hernias in general, have been discussed in meta-analyses<sup>119</sup>. In specific relation to parastomal hernias, overall recurrence rates for open surgery range from 0 – 29% at up to 7 years, and for laparoscopy 0 – 73% at up to 6 years. The majority of the reported open and laparoscopic techniques have differed in regard to the type of mesh employed, relationship of the mesh to stoma, and mesh fixation techniques. Unfortunately, it is impossible to determine from the literature any meaningful comparisons between these strategies as yet again almost all the reports are retrospective case series of small numbers, with mostly limited follow-up (see Table 1.4). Of the mesh application techniques, the two most commonly described include: the ‘Sugarbaker’ intraperitoneal onlay (IPOM) or ‘non-slit’ technique, and the ‘keyhole’ or ‘slit’ method. The former involves fixing the colon to the lateral abdominal wall, then covering the abdominal wall aperture (through which the stoma still passes) and lateralised colon with mesh, thereby creating a flap valve around the stoma<sup>110</sup>; the latter simply involves passing the stoma through an opening in the mesh<sup>111</sup>. One surgical group has reported the use of a combined ‘sandwich’ technique, whereby the bowel is brought through a ‘slit’ in one mesh, followed by an onlay placement of a larger mesh that lateralises the stoma loop<sup>76</sup>. The proposed benefits of the IPOM are that the structural integrity of the mesh is not compromised, unlike that observed when the mesh is ‘slit’<sup>59</sup>, and by creating an oblique tunnel (akin to the inguinal canal), ‘direct’ herniation through a mesh aperture can be avoided; a number of authors have reported comparatively

low recurrence rates with this method<sup>83;114;116</sup>. Nonetheless, this technique can lead to a novel form of bowel obstruction, in which the stoma loop (as it is lateralised on the abdominal wall) is extrinsically stenosed by the edge of the mesh<sup>76;83;114</sup>; the risk of this occurrence is partly related to the tightness of the mesh fixation technique around the lateralised bowel and the acuteness of its resultant angulation. Additionally, the technique (and tightness) of mesh fixation must strike a balance between extrinsic bowel compression and the potential for a loop of bowel to 'indirectly' herniate between the mesh and abdominal wall. Mesh fixation techniques include tacking the mesh to the dorsal layers of the anterior abdominal wall alone, or in combination with transfascial sutures or suturing the mesh to bowel serosa<sup>112;115</sup>; a recent review article found no difference in reherniation or other complication rates between the methods of fixation<sup>120</sup>. Another potential concern of the IPOM technique is that more mesh is in contact with the bowel than with the 'keyhole' technique, increasing the risk of mesh-related complications, although to date this has not been borne out by the literature. The overall mesh-related complication rate ranges from 0 – 28%, and examples include mesh infection, bowel obstruction secondary to mesh-related adhesions and enterocutaneous fistula formation<sup>83;108;111;112;115</sup>. Attempts to reduce this have resulted in a number of different synthetic (including polypropylene, expanded PTFE and polyvinylidene fluoride, PVDF) and biological materials (acellular cross-linked porcine dermal collagen, Permacol<sup>®</sup>, and acellular porcine small intestinal submucosa, Surgisis<sup>®</sup>) being employed, although no appreciable difference in outcome can yet be specifically attributed to any one of them.

**Table 1.4** Published results for intraperitoneal mesh repair of parastomal hernias

Author	Year	Surgical approach	No. of patients	Mesh	Mesh to stoma relation	Mesh fixation	Follow-up (months)	Recurrence (%)	Other complications
Sugarbaker <sup>110</sup>	1985	Open	7	Polypropylene	Non-slit	?	48 - 84	0	?
Morris-Stiff <sup>108</sup>	1998	Open	7	Polypropylene	Slit	?	81	2 (29%)	<ul style="list-style-type: none"> <li>• 1 bowel obstruction</li> <li>• 2° mesh related adhesions</li> <li>• 1 mesh infection</li> </ul>
Voitk <sup>118</sup>	2000	Laparoscopic	4	Polypropylene	?	?	2 - 12	0	0
Kozlowski <i>et al</i> <sup>113</sup>	2001	Laparoscopic	4	ePTFE	?	?	2 - 33	0	0
Safadi <sup>117</sup>	2004	Laparoscopic	9	ePTFE	Slit	Transfascial sutures & tacks	6 - 33	4 (44%)	<ul style="list-style-type: none"> <li>• 1 stoma prolapse</li> </ul>
Stelzner <i>et al</i> <sup>109</sup>	2004	Open	20	ePTFE	Non-slit	Transfascial & peritoneal sutures	3 - 84	3 (15%)	<ul style="list-style-type: none"> <li>• 1 seroma</li> <li>• 1 wound infection</li> </ul>
Van Sprundel <i>et al</i> <sup>111</sup>	2005	Open	16	ePTFE	Slit	Transfascial & peritoneal sutures	5 - 52	1 (6%)	<ul style="list-style-type: none"> <li>• 1 stoma prolapse</li> <li>• 1 hernia between mesh &amp; abdominal wall</li> <li>• 1 E/C fistula</li> </ul>

Author	Year	Surgical approach	No. of patients	Mesh	Mesh to stoma relation	Mesh fixation	Follow-up (months)	Recurrence (%)	Other complications
LeBlanc <i>et al</i> <sup>114</sup>	2005	Laparoscopic	12	ePTFE	5 slit† 7 non-slit	Transfascial sutures & tacks	3 – 39	1 (20%) 0	• 1 seroma • 1 bowel obstruction‡
Ballas <i>et al</i> <sup>75</sup>	2006	Open	2	ePTFE	Slit	Peritoneal sutures	24 – 60	0	0
Hansson <i>et al</i> <sup>112</sup>	2007	Laparoscopic Converted to open	47 8	ePTFE	Slit	Peritoneal & bowel serosal sutures	2	0 1 (12.5%)	• 1 epigastric artery bleed • 1 bowel enterotomy • 1 mesh infection • chronic seromas
Mancini <i>et al</i> <sup>115</sup>	2007	Laparoscopic	25	ePTFE	Non-slit	• Transfascial & bowel serosal sutures • Tacks	2 – 38	1 (4%)	• 1 wound infection • 1 mesh infection
Inan <i>et al</i> <sup>79</sup>	2007	Laparoscopic	2	Porcine dermal collagen (Permacol™)	?	?	3 – 9	0	0
Muysoms <i>et al</i> <sup>116</sup>	2008	Laparoscopic	24	?	11 slit 13 non-slit	?	Mean 31 Mean 14	8 (73%) 2 (15%)	? ?

Author	Year	Surgical approach	No. of patients	Mesh	Mesh to stoma relation	Mesh fixation	Follow-up (months)	Recurrence (%)	Other complications
Craft <i>et al</i> <sup>83</sup>	2008	Laparoscopic	21	ePTFE	5 slit 16 non-slit	Transfascial sutures & tacks	3 -36	1 (20%) 0	<ul style="list-style-type: none"> <li>• 2 mesh infections</li> <li>• 1 wound infection</li> <li>• 2 bowel obstructions<sup>‡</sup></li> </ul>
Zacharakis <i>et al</i> <sup>81</sup>	2008	Laparoscopic	4	ePTFE	Slit	?	Median 9	1 (25%)	0
Berger & Bientzle <sup>76</sup>	2008	Laparoscopic	47	PVDF	'Sandwich' technique	Transfascial sutures & tacks	Median 20	1 (2%)	<ul style="list-style-type: none"> <li>• 2 bowel obstructions<sup>‡</sup></li> <li>• 2 wound infections</li> </ul>
Franklin <i>et al</i> <sup>77</sup>	2008	Laparoscopic	2	SIS	Non-slit	Transfascial sutures & tacks	Mean 52	0	?

E/C Enterocutaneous fistula

ePTFE Expanded polytetrafluoroethylene (Gore-Tex® DualMesh® Biomaterial)

PVDF Polyvinylidene fluoride (Dynamesh IPOM®)

SIS Porcine Small Intestinal Submucosa (Surgisis®)

† 2 'slit' patches used. After encircling the stoma, the slit in the first mesh is suture closed; a second mesh, with slit on the opposite side, is placed over first mesh to provide coverage of the slit in initial mesh.

‡ Angulation of colon secondary to 'non-slit' technique, stenosed the bowel causing obstruction.

\* Sandwich technique employs 2 meshes: the first mesh has a slit, which is suture closed around the stoma; a second larger mesh, is placed over the first and the stoma loop is lateralised

#### **1.2.6.2.1.4 Prevention of parastomal hernias**

Mesh repair of parastomal hernias has now become the gold standard<sup>105;106;121</sup> despite the lack of randomised controlled studies confirming its efficacy over non-mesh repair techniques. In 2003, Carne *et al* calculated an overall hernia recurrence of 7.8% for mesh repairs, by pooling all published results in series containing more than 3 patients<sup>33</sup>, and this has since been cited in over 20 peer-reviewed publications on the subject. By adopting the same approach, the recurrence rate is currently 10% (42 recurrences in 415 mesh repairs) at up to 7 years, and recognition of this, in conjunction with the associated morbidity and economic concerns of a second procedure, has prompted certain surgeons to propose that prevention of parastomal hernias may be the best approach<sup>33;35;56</sup>. To date, 6 surgical groups have reported encouraging results regarding the prophylactic placement of mesh in an attempt to reduce the rate of parastomal herniation<sup>96;122-127</sup> (see Table 1.5). The one randomised controlled trial employed a partially absorbable light-weight polypropylene mesh (Vypro<sup>®</sup>), and demonstrated a 5% incidence of parastomal hernia formation in the treatment (mesh enforced stoma) arm compared to an incidence of 50% in the control (conventional stoma) arm at 12 months. There were no reported mesh-related complications, and although the initial results show the potential of such a strategy, the trial was stopped before statistically pre-determined numbers needed to treat were achieved<sup>96;125</sup>. Additionally, although the herniation rate in the control arm may reflect the experience of some surgeons, it is high compared with published results<sup>33</sup>. The remaining studies are prospective<sup>122;123;127</sup> and retrospective<sup>96;98</sup> case series, which employ either polypropylene (of variable weight and pore size; with or without vicryl)<sup>96;123;125-127</sup> or PVDF<sup>122</sup> mesh, sited in the fascial onlay<sup>96;123</sup>, pre-peritoneal<sup>98;125-127</sup> or intra-peritoneal positions (the same

author combined results for open and laparoscopic approaches)<sup>122</sup>. The overall incidence of parastomal herniation ranges from 0 – 8% (2.2 % incidence, 3 hernias in 134 procedures, from pooled results) at up to 4 years, with a similar range for mesh-related complications. Despite the lack of uniformity in materials and surgical methods, to date the low incidence of parastomal hernias in these studies compared to that reported for conventional stomas makes a strong case for the prophylactic placement of mesh at the time of stoma formation. However, in view of the mesh-related complications reported in both treatment and prevention studies, sufficiently powered randomised controlled trials, with long-term follow up, are needed to determine the best type of material and their most suitable anatomical location for this purpose<sup>34;127</sup>.

**Table 1.5** Published results for prophylactic placement of mesh to prevent parastomal hernias

Author	Year	Study design	No. of patients	Surgical approach	Mesh	Mesh position	Follow-up (months)	Incidence of parastomal hernia (%)	Other complications
Bayer <i>et al</i> <sup>96</sup>	1986	Retrospective case-series	36	Open	Polypropylene (Marlex)	Fascial Onlay	Up to 48	0	<ul style="list-style-type: none"> <li>• 1 stoma stenosis → mesh removed</li> <li>• 4 wound infections</li> </ul>
Janes <i>et al</i> <sup>125,126</sup>	2004	Prospective, randomised controlled	21 mesh 26 no mesh	Open	Composite (Vypro®) <sup>†</sup>	Pre-peritoneal	12	1 (5%) 13 (50%)	0
Israelsson <sup>98</sup>	2005	Retrospective case-series	13	Open	Composite (Vypro®) <sup>†</sup>	Pre-peritoneal	3 – 25	0	<ul style="list-style-type: none"> <li>• 1 wound infection</li> </ul>
Gogenur <i>et al</i> <sup>123</sup>	2006	Prospective case-series	24	Open	Polypropylene (StomaMesh™) <sup>‡</sup>	Fascial Onlay	2 – 26	2 (8%)	<ul style="list-style-type: none"> <li>• 2 meshes eroded through skin</li> </ul>
Marimuthu <i>et al</i> <sup>127</sup>	2006	Prospective case-series	18	Open	Polypropylene (Surgipro™)	Pre-peritoneal	6 – 28	0	<ul style="list-style-type: none"> <li>• 1 wound infection</li> </ul>
Berger <sup>122</sup>	2008	Prospective case-series	25	6 lap 19 open	PVDF (Dynamesh IPST®) <sup>*</sup>	Intra-peritoneal 'keyhole'	2 – 29	0 0	0

† Composite mesh (Vypro®): Polyglactin 910 (vicryl) and large-pore lightweight polypropylene

‡ StomaMesh™: Polypropylene mesh with 2cm wide central ring prepared with lasercut technique

\* PVDF (Dynamesh IPST®): Mesh structure warp-knitted by polyvinylidene fluoride, with polypropylene on parietal surface. Sheet structure with a central hole & funnel (2cm diameter)



#### 1.2.6.2.2 The ideal mesh

The ideal material for the management of parastomal hernias should have adequate strength for the intended surgical application, degrade in parallel with appropriate tissue regeneration, and be capable of sterilization. It also should be non-carcinogenic and relatively inert, causing minimal acute or chronic inflammation (biocompatibility)<sup>128-131</sup>. More specifically, and from a surgeons' perspective, it also should possess the following qualities<sup>128;132</sup>:

- Surgeon friendly handling characteristics;
- Resistance to bacterial colonisation and chronic infection;
- Readily available at acceptable cost;
- Promote parietal tissue in-growth, whilst preventing adhesion to bowel;
- Avoidance of mesh contraction, fistula formation, chronic pain and seroma formation.

Additionally, in those patients prone to hernias, likely secondary to deranged collagen metabolism<sup>50;51;53;133-135</sup>, then either a more permanent structure (rather than one that is completely replaced by host tissue) or one able to correct the balance of collagen metabolism will be required for their management<sup>128</sup>.

### 1.2.6.2.3 Mesh materials

Currently there are more than 70 meshes available for hernia repair on the market<sup>132</sup>, which are either synthetic or derived from biological sources.

#### 1.2.6.2.3.1 **Synthetic mesh**

Synthetic mesh can be permanent or absorbable.

##### *1.2.6.2.3.1.1 Permanent mesh*

Permanent mesh can be classified according to type of mesh, filament structure (monofilament and multifilament), composition and pore size<sup>136</sup>. They are manufactured from one of three basic prosthetic materials: monofilament polypropylene (PP), multifilament polyester (PE), and expanded polytetrafluoroethylene (ePTFE)<sup>132</sup>. Monofilament mesh offers the advantages of high tensile strength and resistance to bacterial attachment but at the expense of decreased pliability and conformity to the abdominal wall<sup>137</sup>. Multifilament mesh is relatively more pliable but is also more susceptible to bacterial infection<sup>138</sup>. Pore size can be described as macroporous (>75µm) or microporous (<75µm). Macroporous mesh allows greater tissue in-growth, and therefore improved biocompatibility but can also promote adhesiogenesis<sup>139;140</sup>, whereas microporous mesh tends to become encapsulated thereby causing less adhesions<sup>132</sup>, but is associated with higher rates of infection<sup>136</sup>. This is explained by the bacterial adhesion and penetration of the small pores that cannot be accessed by leucocytes, thus offering protection from immunological clearance<sup>132</sup>.

The original synthetic meshes were made of a heavyweight woven macro-porous, monofilamentous polypropylene (Marlex®), or multi-filamentous polyester, polyethylene terephthalate (Dacron®)<sup>141;142</sup>. Manufacturers changed the weaves to a

knitted format following complaints of the ends of the mesh unravelling, and these now form some of the more commonly known meshes in use today, such as Prolene<sup>®</sup>, Surgipro<sup>®</sup> and Mersilene<sup>®</sup><sup>137;143</sup>. They work by strengthening the abdominal wall by providing innate mechanical tension and by induction of a strong chronic inflammatory foreign body response<sup>144</sup>. This consequently results in mesh contraction (by 30–50%, usually within 4-weeks of implantation) and formation of an avascular fibrotic conglomerate with the potential for chronic pain and infection, bowel adhesions, visceral erosion, and fistula formation<sup>143;145;146</sup>. Lighter-weight macroporous designs (interwoven with absorbable vicryl (Vypro<sup>®</sup>), or monocryl (Ultrapro<sup>®</sup>), for improved handling) have been shown to reduce the degree of inflammatory response, thereby significantly improving (but not completely removing) the incidence of these complications, without compromising the strength of repair<sup>137;147</sup>. In view of the inflammatory response incited, despite the advent of more biocompatible formats, certain authors advise that surgeons remain wary of employing these materials in close proximity to bowel<sup>147</sup>. Alternatives include ePTFE, and composite meshes, which are composed of a polypropylene or polyester parietal layer and a relatively inert visceral surface.

Expanded PTFE (Gore-Tex<sup>®</sup>, MycoMesh<sup>®</sup> and DualMesh<sup>®</sup>) is relatively inert and microporous, therefore, it tends not to instigate as vigorous an inflammatory response as polypropylene or become incorporated into host tissues<sup>148</sup>. Instead, ePTFE becomes encapsulated, which confers the advantage of minimizing intestinal adhesions, thus allowing for placement in close proximity to bowel<sup>149-151</sup>. However this benefit is off-set by a potentially weaker hernia repair and a higher prevalence of infective complications compared to all other mesh types<sup>143</sup>. Encapsulation and the

microporous character of ePTFE means antimicrobials and the host immune system have reduced accessibility to microorganisms<sup>152</sup>. Therefore, when ePTFE meshes become infected they must be removed, and as such their use cannot be recommended in contaminated or potentially contaminated fields<sup>132;152;153</sup>. Attempts to improve tissue integration, by creating full-thickness pores (MycoMesh<sup>®</sup>) and roughening the parietal surface (DualMesh<sup>®</sup>), as well as the addition of an antimicrobial silver chlorhexidene film (DualMesh Plus<sup>®</sup>), have not yet been proven to reduce the incidence of the above stated concerns<sup>154</sup>.

Composite meshes were developed in response to the increasing popularity of intraperitoneal placement of mesh for incisional hernia repair and its associated challenges. The manufacturers' aims were to produce a mesh with a visceral surface, which protects the bowel and avoids adhesion formation, and a parietal surface that promotes host tissue integration. Different strategies have been used to achieve this goal. Composix<sup>®</sup> (Bard) and Dynamesh<sup>®</sup> (FEG Textiltechnik) place polypropylene against the abdominal wall for strong in-growth, and PTFE or PVDF (polyvinylidene fluoride) respectively, toward the bowel to minimise adhesion formation. Proceed<sup>®</sup> (Ethicon) and SepraMesh<sup>®</sup> (Genzyme) also use polypropylene for strong incorporation to the abdominal wall but coat the material with a resorbable cellulose-based material. Similarly, Parietex Composite<sup>®</sup> (Sofradim) places a resorbable collagen-oxidised film onto a polyester mesh base. The resorbable layer provides a temporary barrier between the mesh and viscera, which need to be protected for 7 – 14 days. Evidence suggests that adhesion formation to the bowel occurs in the first week after surgery, after which a neoperitoneum covers the mesh and provides long-term protection<sup>143;147;155</sup>. Studies have shown that despite the use

of these composite materials, adhesions and bowel-related complications still occur<sup>143;151;156</sup>. It is possible these are related to the mesh fixation technique and/ or differential in contraction between the inflammatory parietal and relatively inert visceral layers, which leads to a rolling of the mesh edges and thus exposure of the polypropylene or polyester to the bowel<sup>147</sup>.

#### *1.2.6.2.3.1.2 Absorbable mesh*

Absorbable meshes, such as those made of polyglactin 910 (Vicryl) and polyglycolic acid (Dexon), have the advantage of an improved host tissue response, low risk of chronic infection and avoidance of bowel related complications. Theoretically, they should provide acute support to the abdominal wall defect, followed by degradation in parallel with new fibro-connective tissue, which should take over the functional repair. However, long-term follow up data indicates that in terms of hernia recurrence, absorbable mesh repair has no benefit over simple suture repair<sup>152;157</sup>, and therefore when used alone, their role cannot be justified in the management of abdominal wall hernias.

#### **1.2.6.2.3.2 Biological mesh**

These are harvested from animals (xenogeneic), usually porcine or bovine sources, or humans (allogenic). They are rendered acellular via a variety of methods to provide a biocompatible scaffold for host cell population, vascularisation and eventually complete soft tissue repair. They can potentially therefore avoid acute and chronic mesh infection, or exaggerated host immune response and its sequelae, seen in response to permanent synthetic meshes. However, premature enzymatic degradation of the specific extracellular matrix (ECM) graft components can lead to

graft resorption before adequate tissue in-growth has occurred, and consequently rates of reherniation not dissimilar to those observed with absorbable synthetic grafts<sup>158-161</sup>. Methods of impeding the rate of resorption, such as chemical cross-linkage, have been shown to significantly improve the rate of hernia recurrence<sup>159;161;162</sup>, although possibly at the expense of host tissue integration.

Three commercially available biological tissue grafts have been used for ventral hernia repair, including parastomal hernias, of which two are biodegradable: porcine small intestinal submucosa (Surgisis<sup>®</sup>), and human cadaveric dermis (AlloDerm<sup>®</sup>); and one is cross-linked: porcine dermal collagen (Permacol<sup>®</sup>). To date, there have been no reports of disease transmission from any of these products to their recipients.

#### *1.2.6.2.3.2.1 Surgisis<sup>®</sup> (SIS)*

SIS consists of an acellular non-cross-linked ECM sheet, derived from porcine small intestinal mucosa. It is composed of over 90% collagen (in particular subtypes I, III and V), and 10% glycoproteins, proteoglycans, glycosaminoglycans and lipids<sup>163</sup>. Available as a 4 or 8-layer product, the latter (Surgisis Gold<sup>®</sup>) is recommended for abdominal wall repair<sup>128</sup>, on account of its greater mechanical strength than both the 4-layer product and natural abdominal wall fascia<sup>164</sup>. Animal models have shown that when implanted into the abdominal wall SIS invokes a limited host inflammatory response, with evidence of graft neovascularisation (> 50% thickness of the implant at 8-weeks) and deposition of well-organised host connective tissue at 90 days<sup>128</sup>. In a rodent model, when compared to polypropylene, there was significantly less foreign-body response, consequently less adhesion formation, and collagen

deposition was better organised<sup>164-168</sup>. SIS is biodegradable, and crucial to its value as an abdominal wall repair material is its speed of degradation, the comparative rate of host remodelling and the quality and strength of the newly formed host tissue. Animal studies have shown that SIS is 25% histologically absent at 1 month, increasing to 100% absent at 4 months when used to repair abdominal wall defects in canine models<sup>164</sup>. In clinical studies, accelerated degradation has been reported when SIS is used to reconstruct abdominal wall defects in contaminated fields, which the authors hypothesized was responsible for early hernia recurrence<sup>169;170</sup>. Experimental studies have however shown that the infectivity of inoculated wounds implanted with SIS was significantly less compared with a permanent synthetic material at 28 days<sup>166</sup>.

Although SIS has reportedly only been used in 2 patients with parastomal hernias<sup>77</sup> (no hernia recurrence at 2 years, see Table 1.4), there are a number of studies that have evaluated its usefulness for incisional hernia repair in clean, potentially contaminated and contaminated wounds<sup>77;169-171</sup>. At up to 2½ years follow up, recurrence rates for clean and potentially contaminated wounds have been reported at up to 10%, and for contaminated wounds range from 30-50%. A relatively high rate of seroma formation and post-operative pain has also been reported<sup>172</sup>, and this is because serous infiltrate accumulates between the laminated layers of SIS, thought to be related to the absence of pores and therefore limited capacity for fluid to flow through the material<sup>77;170</sup>.

#### 1.2.6.2.3.2.2 AlloDerm®

AlloDerm® is an acellular non-cross-linked allogenic tissue graft, derived from human cadaveric skin. As the manufacturers rely on donors, its availability is presumably relatively limited, and this is reflected in a cost price at least three times that of those biological grafts harvested from xenogeneic sources<sup>147</sup>. It is composed of a structurally intact vascular basement membrane, collagen fibres (subtypes I, III, IV and VII), elastin filaments, laminin and glycosaminoglycans<sup>128</sup>. Clinical and animal studies have shown that AlloDerm® does not induce a chronic inflammatory response or induce visceral adhesions, that *in vivo* full-thickness fibrovascular integration occurs by at least 8 months<sup>173;174</sup>, and that complete implant degradation can be prolonged (in a porcine model, implant constituents could be detected 9 months after implantation)<sup>174</sup>. Experimental studies have shown that AlloDerm® has no more intrinsic resistance to pathogens than Surgisis® or a number of synthetic meshes<sup>166;175</sup>. Clinically, AlloDerm® has been used to treat both parastomal and incisional ventral hernias, and notwithstanding the small number of patients, the varying anatomical sites of AlloDerm® implantation and the limited follow-up, the rates of hernia recurrence are up to 37% for parastomal hernia repair<sup>74;94</sup> (see Table 1.2) and up to 50% for infected or potentially infected incisional hernias<sup>176-178</sup>. When compared to synthetic mesh (PTFE or woven polyethylene), there was a higher incidence of mesh-related complications and hernia recurrence (4.5% vs 13%) in the synthetic mesh than AlloDerm® arms<sup>128</sup>. However, in comparison to Surgisis®, rates of recurrence were considerably higher in the AlloDerm® cohort (0% vs 24%)<sup>172</sup>. Interestingly, unlike synthetic mesh which contracts over time, AlloDerm® thins out and stretches<sup>173;179</sup>, which may at least partly account for the relatively high incidence of hernia recurrence associated with its use. Authors have also reported



significant time-consuming effects of using AlloDerm<sup>®</sup>, as it is only available in small sheets and therefore has to be 'quilted' to cover large defects<sup>128</sup>.

#### 1.2.6.2.3.2.3 Permacol<sup>®</sup>

Permacol<sup>®</sup> is an acellular isocyanate cross-linked collagen sheet derived from porcine dermis. It is composed of over 90% type I collagen, with type III collagen and elastin fibres comprising the remainder. The implant contains naturally occurring pores, in the form of hair follicle remnants, which according to the manufacturer are 254 - 654µm in diameter. The suitability of Permacol<sup>®</sup> to act as a bioconstruct for soft tissue reinforcement and repair has been investigated both *in vitro* and in animal models. *In vitro* studies have shown that Permacol<sup>®</sup> supports cell attachment, growth and stratification, does not inhibit cellular proliferation, and that the cross-linking confers resistance to collagenase degradation, albeit with a corresponding reduction in cellular infiltration<sup>180;181</sup>. When implanted into rats, Permacol<sup>®</sup> induced a mild chronic inflammatory response with no evidence of significant fibrosis, and cellular infiltration and neovascularization limited to its peripheries and native pores<sup>182-186</sup>. In contrast to polypropylene, comparative studies in animal models have demonstrated that Permacol<sup>®</sup> has better tissue compatibility, with less intraperitoneal adhesion formation, more orderly collagen deposition and comparable tensile strength at the interface between the implant and host tissue, at 90 days after implantation<sup>182;183</sup>. Interestingly, two animal studies have compared Permacol<sup>®</sup> and Surgisis<sup>®</sup>, and although they showed no difference in the degree of host chronic inflammation or adhesion formation<sup>185;187</sup>, Surgisis<sup>®</sup> demonstrated a significantly greater degree of fibrovascular integration and collagen deposition at 9 weeks<sup>187</sup>, although there was no difference at 20 weeks<sup>185</sup>. Experimental hernia models comparing Permacol<sup>®</sup> and

Alloderm<sup>®</sup> demonstrated no significant differences in adhesion formation, or cellular in-growth and neovascularisation at 3 months, but at 6 months whereas Permacol<sup>®</sup> continued to provide a robust repair, Alloderm<sup>®</sup> had lost tensile strength and was associated with hernia-like bulging<sup>179</sup>.

To date, there is only one published case report on the use of Permacol<sup>®</sup> to repair parastomal hernias<sup>79</sup> (see Table 1.4), however a comprehensive Medline search (combining the keywords: Permacol<sup>®</sup>, collagen, ventral, incisional and hernia; and limited to English language papers) identified 11 articles reporting the clinical use of Permacol<sup>®</sup> to repair anterior abdominal wall defects in general (See Table 1.6). Two of these reported the use of Permacol<sup>®</sup> to close the abdominal wall in paediatric renal transplant recipients, to avoid compartment syndrome, and therefore any inferences drawn in regards to adult hernia repair or prevention are limited<sup>188;189</sup>. Hernia recurrence in the remaining studies, all of which were performed (with the exception of 2 cases<sup>190</sup>) in clean-contaminated or contaminated wounds, ranges from 0 – 15% at up to 18-months follow up (overall incidence 7.2%; 10 recurrent hernias in 137 repairs). The other mesh related complications included 1 mesh infection requiring its removal and an overall incidence of 7.2% chronic seroma formation.

**Table 1.6** Published results of Permacol® mesh repair of anterior abdominal wall defects

Author	Year	Study Design	No. of patients	Wound Type	Follow-up (months)	Recurrence (%)	Other complications
Adedeji <i>et al</i> <sup>191</sup>	2002	Case-report	1	<ul style="list-style-type: none"> <li>• Clean-contaminated</li> </ul>	12	0	Nil
Verey <i>et al</i> <sup>192</sup>	2004	Retrospective case-series	10	<ul style="list-style-type: none"> <li>• 2 contaminated</li> <li>• 8 clean-contaminated</li> </ul>	2 – 11	0	<ul style="list-style-type: none"> <li>• 2 wound infections</li> <li>• 1 removal of implant 2° adhesions</li> </ul>
Richards <i>et al</i> <sup>189</sup>	2005	Retrospective case-series	3	<ul style="list-style-type: none"> <li>• Clean</li> </ul>	18	0	Nil
Cobb & Shaffer <sup>193</sup>	2005	Retrospective case-series	60	<ul style="list-style-type: none"> <li>• 5 contaminated</li> <li>• 56 clean-contaminated</li> </ul>	Mean 14	4 (6.6%)	<ul style="list-style-type: none"> <li>• 2 wound infections</li> <li>• 2 chronic seromas</li> </ul>
Liyanage <i>et al</i> <sup>194</sup>	2006	Case-report	1	<ul style="list-style-type: none"> <li>• Clean-contaminated</li> </ul>	12	0	<ul style="list-style-type: none"> <li>• Seroma</li> <li>• Superficial wound dehiscence</li> </ul>
Parker <i>et al</i> <sup>190</sup>	2006	Retrospective case-series	9	<ul style="list-style-type: none"> <li>• 2 clean</li> <li>• 2 clean-contaminated</li> <li>• 5 contaminated</li> </ul>	Median 18	0	<ul style="list-style-type: none"> <li>• 1 mesh sepsis → removal of mesh</li> <li>• 1 superficial wound dehiscence</li> </ul>
Saettele <i>et al</i> <sup>195</sup>	2007	Case-report	1	<ul style="list-style-type: none"> <li>• Contaminated</li> </ul>	12	0	<ul style="list-style-type: none"> <li>• Chronic seroma</li> </ul>

Author	Year	Study Design	No. of patients	Wound Type	Follow-up (months)	Recurrence (%)	Other complications
Catena <i>et al</i> <sup>196</sup>	2007	Prospective case-series	7	• Contaminated	Mean 11	0	Nil
Shaikh <i>et al</i> <sup>197</sup>	2007	Retrospective case-series	20	• Clean-contaminated	Median 18	3 (15%)	<ul style="list-style-type: none"> <li>• 2 seromas</li> <li>• 2 wound infections</li> <li>• superficial wound dehiscence</li> </ul>
Pentlow <i>et al</i> <sup>188</sup>	2008	Retrospective case-series	5	• Clean	Up to 36	1 (20%)	<ul style="list-style-type: none"> <li>• 1 superficial wound dehiscence</li> </ul>
Hsu <i>et al</i> <sup>198</sup>	2008	Retrospective case-series	28	• Clean-contaminated	Mean 16	3 (10.7%)	<ul style="list-style-type: none"> <li>• 1 superficial wound dehiscence</li> <li>• 1 wound infection</li> <li>• seromas</li> </ul>

### 1.2.7 Conclusion

In contrast to published data relating to conventional synthetic implants, there have been no reported complications related to the proximity of these biological implants to the bowel. These benefits, in conjunction with the perceived merits of cross-linkage for biologically derived materials, and the aforementioned advantages of siting mesh at the time of stoma formation, led to the development of a prospective randomised controlled phase 1 study to assess the role of Permacol® in the prevention of parastomal hernias (presented in chapter 2). Histological analyses of explanted implant specimens further permitted evaluation of the *in vivo* human response to its presence.

## 1.3 Anal fistula

### 1.3.1 Introduction

‘Probably more reputations have been damaged by unsuccessful treatment of cases of fistula than by excision of the rectum or gastroenterostomy’

Lockhart-Mummery, 1929

The majority of anal fistula cases can be managed without complication, but a significant minority can present a major challenge to both patient and surgeon. The difficulty in managing this subset of patients was recorded by Hippocrates in 460 BC, and is further emphasized by reports from the middle ages of clinicians whose primary function was to treat anal fistula, and the highest surgical fee in history being paid for treating the fistula of Louis XIV.

Anal fistulas are chronic pathological connections between the anal canal and the skin of the perineum or buttocks, often passing through the anal sphincter complex (responsible for maintaining continence to rectal contents, including gas, liquid and solid stool). They are subject to either persistent discharge or recurrent episodes of pain and swelling (abscess formation), eased by either spontaneous drainage of pus or repeated hospital admissions for surgical drainage. Difficulties in their management are bestowed by their unique anatomical relationship to the anal sphincters. To date the most successful management strategy for the treatment of fistulas involves surgically dividing the tissue enclosed by the fistula tract; however

division of those sphincter muscle fibres enclosed renders the patient at risk of faecal incontinence.

Anal fistulas may be found in association with a variety of specific conditions, but the majority (>90%) seen in the U.K. are classified as non-specific, cryptoglandular or idiopathic, their exact aetiology having never been fully proven, although the diseased anal gland in the intersphincteric space is deemed central<sup>199</sup>. Anal fistulas may be seen in association with Crohn's disease<sup>200</sup>, tuberculosis<sup>201</sup>, pilonidal disease, hydradenitis suppurativa<sup>202</sup>, malignancy<sup>203</sup>, trauma and foreign bodies<sup>204</sup>. The work herein is concerned only with the non-specific form of fistula unless otherwise stated.

### 1.3.2 Epidemiology

Current knowledge of the exact incidence of idiopathic anal fistulas in the general population is scarce. However, the most accurate data is from northern Europe, which indicates an incidence of 8.6-10/ 100,000 population per year<sup>205;206</sup>, and more recently from a study that analysed the incidence of fistula-in-ano (although not specifically of idiopathic aetiology) in four countries of the European Union (England, Spain, Italy and Germany), and reported an incidence of 1.2-2.8/ 10,000 inhabitants per year<sup>207</sup>. Nearly all reported series have shown a male predominance, the male to female ratio being between 2:1 and 4:1<sup>199</sup>. This may partly be explained by the finding that males have more intramuscular anal glands, the presumed aetiological source, than females (1.4:1), and that these are more frequently ramifying and are of a more cystic nature, all of which are factors that might make individuals more susceptible to infection<sup>208</sup>. However this is disputed by McColl who found no sex

differences in histology or distribution of anal glands in 50 normal human canals<sup>209</sup>. Furthermore, no differences in circulating sex hormones between patients, and age and sex-matched controls have been demonstrated<sup>210</sup>.

Anal fistulas most commonly afflict people in their third to fifth decade<sup>208;211;212</sup>. There has been no association found between sedentary occupations, poor personal hygiene or perianal perspiration and the role of bowel habit is unclear. Diarrhoea and constipation have been implicated in the aetiology, in that loose stool may allow the easier passage of pathogens to the anal glands<sup>213</sup>, and hard stool may have a similar effect secondary to anal canal abrasions<sup>214</sup>, but neither theory has been substantiated.

### 1.3.3 Aetiology

An appreciation of the anatomy of the anal canal is crucial to understanding both the cryptoglandular hypothesis<sup>215</sup>, the most widely accepted (albeit never absolutely proven) theory on anal fistula pathogenesis, and its necessarily diverse management.

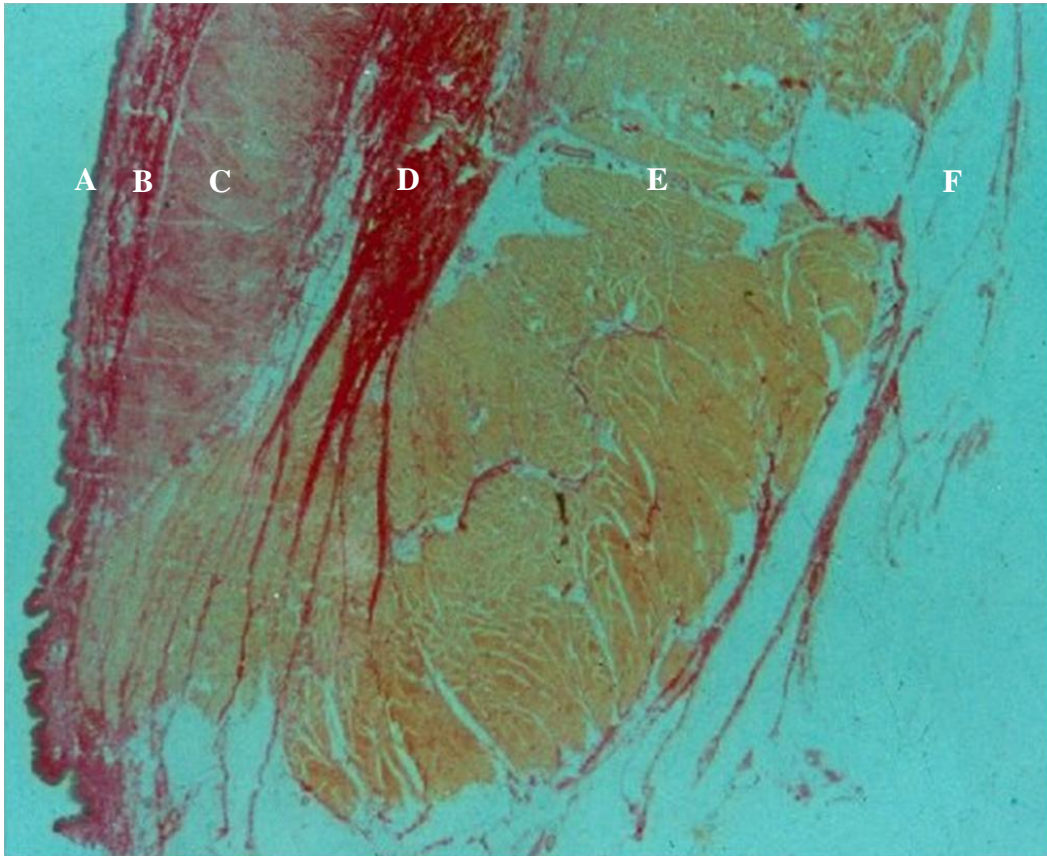
#### 1.3.3.1 Anatomy of the anal canal

The anal canal in adults is approximately 4cm long and begins as the rectum narrows, passing backwards between the levator ani (pelvic floor) muscles, and ending at the anal verge<sup>216;217</sup>. The proximal canal is lined by simple columnar (mucosal) epithelium, which changes to stratified squamous epithelium lower in the canal via an intermediate transition zone just above the dentate line<sup>218</sup>. The dentate line represents the site of anal valves, embryological remnants of the developing foetal hindgut<sup>219</sup>. Deep to the mucosa lies the subepithelial tissue, composed of

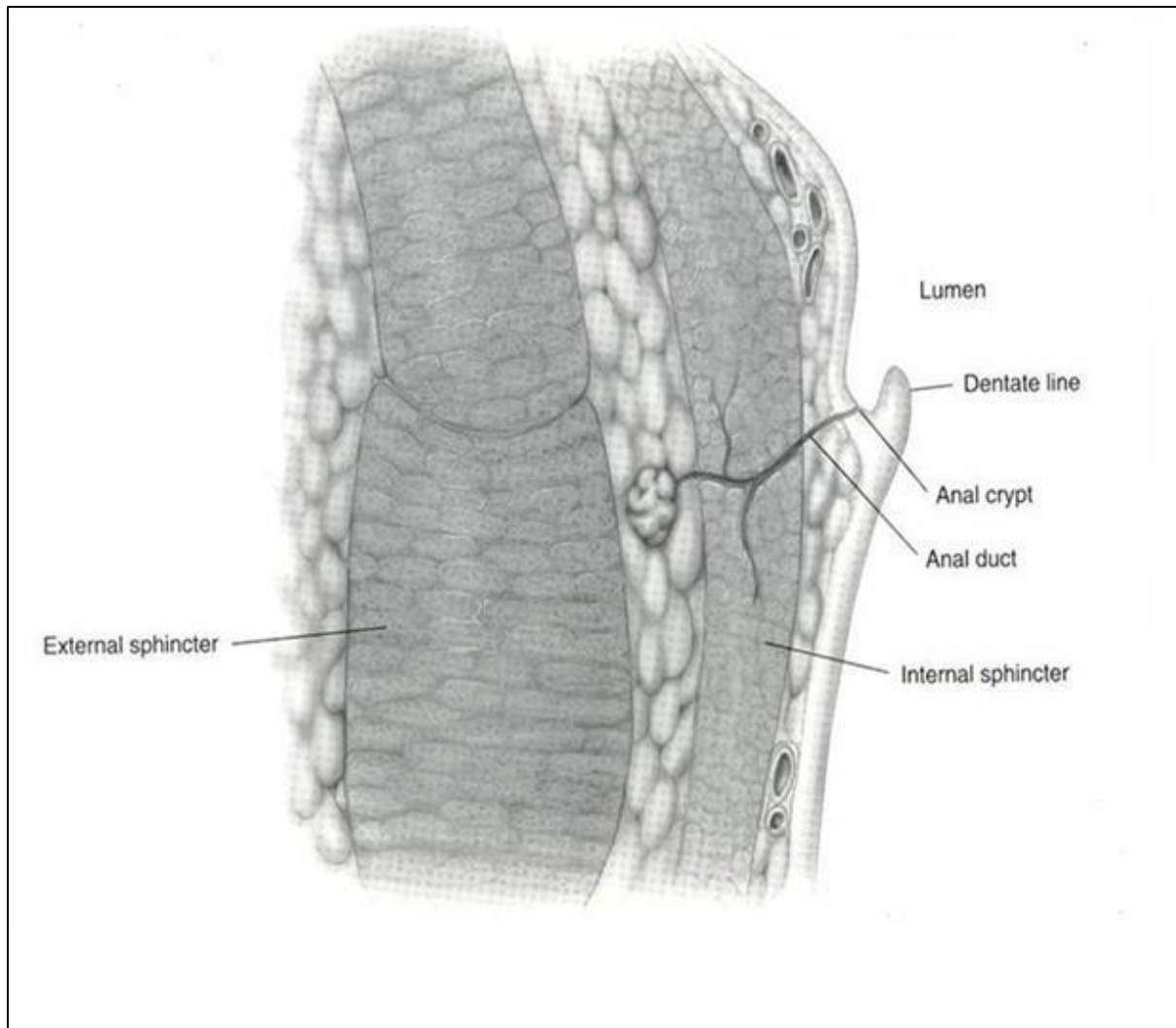


connective tissue and smooth muscle<sup>217</sup>. This forms the basis of the vascular haemorrhoidal cushions, which are important in the maintenance of continence<sup>220;221</sup>. Lateral to the subepithelial layer the caudal continuation of the circular smooth muscle of the rectum forms the internal anal sphincter<sup>218</sup>, which terminates at a variable distance from the anal verge, with a well-defined border. Continuous with the outer layer of the rectum, the conjoint longitudinal muscle of the anal canal lies between the internal and external anal sphincters in the intersphincteric space. The conjoint longitudinal muscle comprises smooth muscle cells from the rectal wall, augmented with striated muscle from a variety of sources, including the levator ani, puborectalis and pubococcygeus muscles of the pelvic floor. Fibres from this layer traverse the intersphincteric space and both internal and external sphincters, inserting distally into the perianal skin, medially to the anal canal mucosa, and fascia laterally at the pelvic side walls, to form a complex supporting meshwork of septa, which anchors the sphincter complex in place. The striated muscle of the external sphincter surrounds the conjoint longitudinal muscle, forming the outer border of the intersphincteric space, and terminates subcutaneously below the caudal limit of the internal anal sphincter<sup>222</sup>. Collectively, the internal and external sphincters, and the interposed conjoint longitudinal muscle fibres, are termed the anal sphincter complex. Lateral to the external sphincter is the ischiorectal fossa, which is composed almost entirely of relatively avascular loose areolar tissue, and is contained anterosuperiorly by the sloping roof of the levator ani muscle, posteroinferiorly by the skin of the buttock, and further laterally by the ischial tuberosity of the pelvis (see Figure 1.2).

Anal glands sit in both the submucosal and intersphincteric spaces, with those situated in the intersphincteric space constituting one to two-thirds of their total<sup>209</sup>. The anal gland ducts traverse the internal sphincter to open into the anal canal lumen, via crypts situated above the anal valves (see Figure 1.3). The function of the anal glands is uncertain, although they have been shown to secrete mucin<sup>223</sup>. The anal glands in the intersphincteric space are central to the cryptoglandular hypothesis of anal fistula formation.



**Figure 1.2** Anatomy of anal sphincter complex. (A) Anal canal mucosa. (B) Submucosal space. (C) Internal anal sphincter (IAS). (D) Conjoint longitudinal muscle fibres, seen transversing the internal and external anal sphincters. (E) External anal sphincter (EAS), terminating subcutaneously below the caudal limit of the IAS. (F) Ischiorectal fossa. This figure is a reprint from reference 250 (reproduced with permission by Mark Allen Healthcare Ltd).



**Figure 1.3** Anatomy of intersphincteric anal gland. The anal gland duct can be seen to traverse the internal sphincter to open into the anal canal lumen, via crypts situated at the dentate line. Picture provided courtesy of Peter J Lunniss, Senior Lecturer & Honorary Consultant in Coloproctology.

### 1.3.3.2 Cryptoglandular hypothesis

Parks hypothesised that intersphincteric gland dilatation, congenital or acquired, was a precursor to mucin accumulation, which was consequently prone to infection from ascending enteric bacteria via the openings of the anal ducts<sup>215</sup>. The infected intersphincteric gland is unable to drain spontaneously back into the anal canal, because of inflammatory obstruction of its connecting duct across the internal sphincter<sup>224</sup>, and thus spreads along planes of least resistance, following the fibres of the conjoint longitudinal muscle, and usually emerges at the skin as an abscess. Parks further proposed that should the initial abscess in relation to the intersphincteric anal gland subside, the diseased gland might become the seat of chronic infection with subsequent fistula formation<sup>215</sup>. The fistula is thus a granulation tissue-lined track maintained by the infecting source, which is the chronically infected anal gland deep to the internal anal sphincter.

The only studies that have employed microbiology in testing the cryptoglandular hypothesis have been unable to demonstrate an abundance of organisms in the intersphincteric space of established fistulas<sup>225;226</sup>. However, it has been shown that persistence of anal fistulas, in a similar fashion to those found at other body sites, may at least be partly due to the in growth of epithelium from either or both ends of the track<sup>227</sup>.

The spread of sepsis from an acutely infected anal gland may occur in the vertical, horizontal or circumferential planes. Caudal spread in the vertical (intersphincteric) plane is the commonest way by which infection disseminates, and presents as a perianal abscess, arising at the anal verge. Cephalad spread will result in an intermuscular or supralelevator pararectal abscess. Horizontal spread across the external sphincter will enter the ischiorectal fossa. Caudal spread in this plane will

lead to an ischiorectal abscess, terminating at the skin of the buttock; upward spread may penetrate the levator ani muscle to reach the supralelevator pararectal space (see Figure 1.4). Circumferential spread may occur in any of the intersphincteric, extrasphincteric or supralelevator planes, in a horse-shoe configuration, with one or more openings onto the ipsilateral or contralateral skin of the perineum or buttocks<sup>199</sup>.

### 1.3.4 Management

Traditionally, the initial management of acute anal sepsis is by simple incision and drainage of the abscess, and further discussion is not within the remit of this thesis. In contrast, the management of chronic anal fistulas is necessarily more diverse, and depends upon accurate anatomical knowledge of the fistula's course through the anal sphincter complex. Failure to appreciate the importance of this relationship may result in fistula recurrence, incontinence or catastrophically both. Classification of the pathology is therefore of the up most importance, as it will guide surgical management.

#### 1.3.4.1 Classification

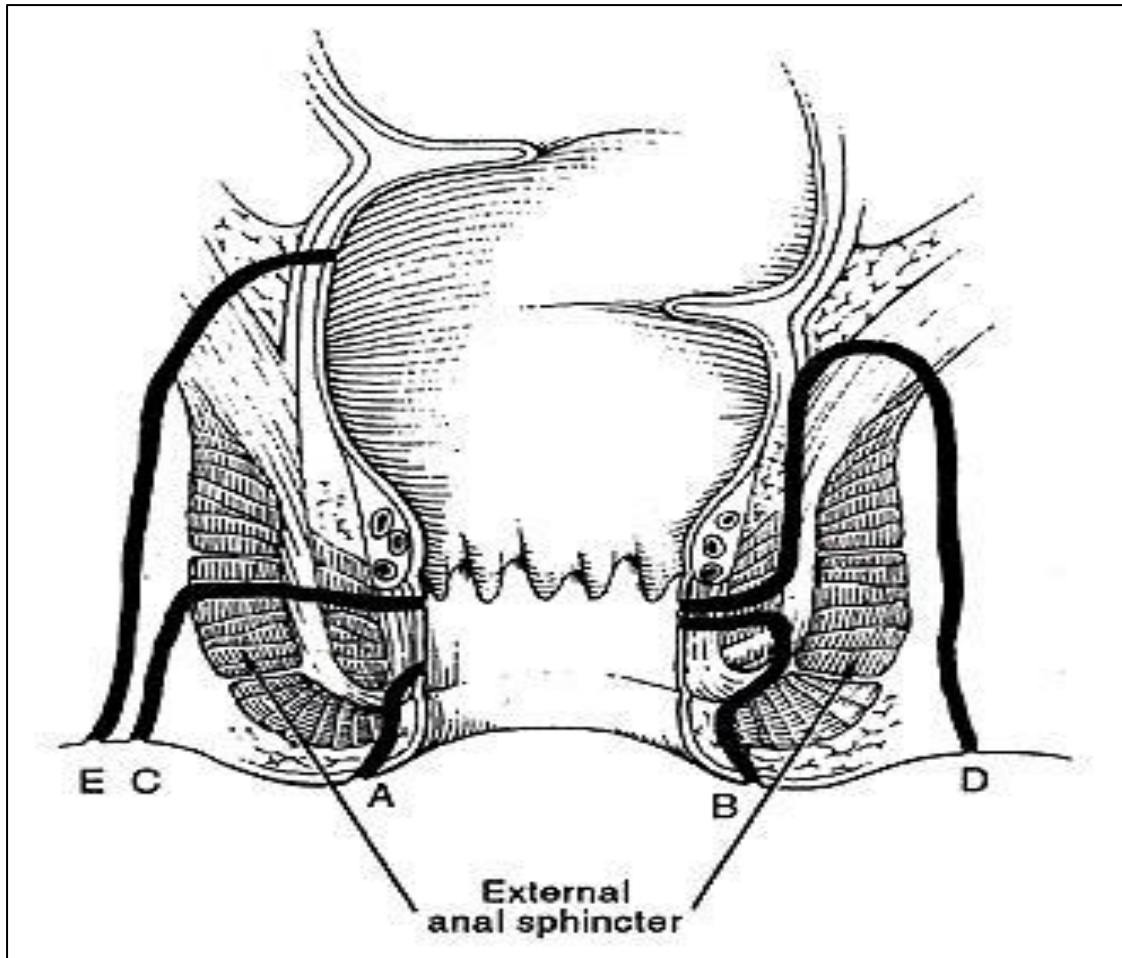
The most comprehensive, practical and widely used classification is that devised by St. Mark's Hospital. It is based on the cryptoglandular hypothesis, and the relationship of the primary fistula track to the external sphincter<sup>228</sup>. Four main groups exist: intersphincteric, transsphincteric, suprasphincteric and extrasphincteric (see Figure 1.4). These groups can be further subdivided according to the presence and course of any extensions or secondary tracks.

Intersphincteric fistulas (45%) are usually simple tracks (uncomplicated fistulas consisting only of the primary track) passing down through the intersphincteric space to the perianal skin; but others may have a high blind track, a secondary high opening into the rectum or no perianal opening, or even a high pelvic extension.

Transsphincteric fistulas (30%) cross the external sphincter to pass through the ischiorectal fossa to reach the skin of the buttocks. They may be subdivided into 'high', 'mid' or 'low' dependant on where the track crosses the external sphincter into the ischiorectal fossa: above, at the level of, or below the dentate line respectively. This may not be at the same level the track crosses the internal sphincter. Fistulas may be simple or have a blind high track terminating above or below the levator ani muscles.

Suprasphincteric fistulas (20%) run up the intersphincteric space to a level beyond the puborectalis and then curl over it through the levator ani and into the ischiorectal fossa to reach the skin. An argument exists as to whether suprasphincteric tracks can be part of this classification, as some believe that they are iatrogenic as opposed to cryptoglandular in nature.

Extrasphincteric fistulas (5%) are not of cryptoglandular pathology. They run without relation to the sphincters and are classified according to their pathogenesis.



**Figure 1.4** Classification of anal fistulas. (A) Submucosal fistula. (B) Intersphincteric fistula. (C) Transsphincteric fistula. (D) Suprasphincteric fistula. (E) Extrasphincteric fistula. This figure is a reprint from reference 250 (reproduced with permission by Mark Allen Healthcare Ltd).



### 1.3.4.2 Assessment

#### 1.3.4.2.1 Clinical

A full history and examination including proctosigmoidoscopy are essential in all cases to assist in determining the aetiology of the fistula. Clinical assessment involves five essential points<sup>229</sup>: location of the internal opening, location of the external opening, the course of the primary track, the presence of any secondary extensions, and the presence of other diseases complicating the fistula.

Digital assessment of the primary track, by an experienced coloproctologist, in the conscious patient has been shown to be 85% accurate<sup>230</sup>. It is further complimented by examination under anaesthesia (EUA), during which a probe can be utilised to delineate the primary track. If not initially evident, the instillation of dilute hydrogen peroxide via the external opening has been advocated as the best agent to identify the internal opening<sup>231</sup>.

#### 1.3.4.2.2 Imaging

Although careful examination under anaesthetic is the most important part of any assessment, previous surgery can lead to scarring and deformity, and complex fistulas with multiple secondary tracks make clinical assessment difficult<sup>230</sup>. In such situations, there is a need for further methods of assessing fistulas and their relationship to the sphincters. Two modalities of imaging have to date proven their usefulness: anal endosonography and magnetic resonance imaging.

Anal endosonography (AES) is safe, simple to perform and relatively inexpensive. Although initial results indicated a high degree of accuracy in delineating the intrasphincteric component of anal fistulas<sup>232</sup>, later studies showed the technique not to be any more accurate than careful digital examination under anaesthesia<sup>230</sup>.

Furthermore, its limited focal range can result in a low positive predicative value in the demonstration of extensions beyond the external sphincter, and thus evaluation of sepsis within the ischiorectal and supralelevator spaces, which limits its usefulness when dealing with complex fistulas<sup>233</sup>. More recent studies however have shown it to be superior to clinical evaluation<sup>234</sup>, and demonstrate good concordance with operative findings<sup>235;236</sup>. Currently, however its main role is in assessing the sphincter complex, specifically in determining internal and external anal sphincter integrity, prior to planning fistula surgery.

Magnetic resonance imaging (MRI) is considered the gold standard in anal fistula imaging<sup>237</sup>, and a number of studies have now confirmed that the technique challenges operative assessment by an experienced coloproctologist<sup>234;238</sup>. Its advantages include the lack of ionising radiation, the ability to image in any plane and the high soft tissue resolution<sup>239</sup>. Short tau inversion recovery (STIR) (a fat suppression technique) sequencing, to highlight the presence of pus and granulation tissue without the need for any contrast media, has a concordance with operative findings of 86% for the presence and course of the primary track, and a positive predicative value of 100% in demonstrating secondary extensions and abscesses, which if missed at surgery would result in fistula recurrence<sup>199;237</sup>. Dynamic contrast-enhanced MR using intravenous gadolinium chelates has been further shown to increase track conspicuity, particularly when fat saturation or subtraction is employed<sup>240;241</sup>. MRI has also been shown to predict recurrence following treatment<sup>242;243</sup>.

#### 1.3.4.2.3 Physiological

Continence may be regarded as a balance between rectal pressure and the power of the sphincters to overcome this, orchestrated by anorectal sensation<sup>199</sup>. Anorectal physiological studies provide measurements of the sphincteric pressures generated along the canal. The internal anal sphincter, made of smooth muscle and not under voluntary control, contributes 60-85% of resting anal pressure<sup>244-246</sup>. Damage to this muscle can result in symptoms of passive anal leakage, including soiling of underwear, and flatus incontinence<sup>247</sup>. The external anal sphincter, under conscious control, contributes to the maintenance of resting pressure, and in association with the puborectalis muscle generates squeeze pressure, the loss of which causes symptoms of urgency (reduced ability to retain rectal contents) and frank faecal incontinence (inability to retain rectal contents)<sup>247</sup>.

Complete division of the puborectalis sling in extrasphincteric and suprasphincteric fistulas would result in total incontinence to all rectal contents. Below this level the term incontinence becomes relative, dependant more on subjective values of the patient (and its impact on individual quality of life) than on objective measurements obtained in a physiology laboratory<sup>199</sup>. However, it is reasonable to assume that the higher the level at which the primary track crosses the sphincter complex (ie. the more sphincter tissue enclosed by the fistula), the greater the possibility of impaired function after surgical division; and the weaker the sphincters before surgical intervention, the greater the likelihood of such morbidity<sup>248</sup>. Preoperative anorectal physiology assessment can be used to identify patients at risk of incontinence<sup>249</sup>, and thus guide surgical treatment, but it does not necessarily predict postoperative physiological or functional outcomes<sup>250</sup>.

Traditionally, fistula surgeons have apportioned more importance to the preservation of the external than the internal sphincter, for the purpose of maintaining continence, and it is still believed amongst many surgeons that division of the internal sphincter, to eradicate the presumed aetiological source (the diseased anal gland in the intersphincteric space), is essential to prevent fistula recurrence. However, it is important to realise that division of the internal anal sphincter, as in fistulotomy for intersphincteric fistulas, may not only result in diminished resting pressures, but may also impact on squeeze pressures in the most distal part of the sphincter complex, on account of the subcutaneous external anal sphincter lying below the caudal limit of the internal anal sphincter.

In a prospective study comparing internal sphincter division alone with both internal and external sphincter division<sup>250</sup>, as part of treatment for intersphincteric and transsphincteric fistulas respectively, there was no difference, 53% versus 50%, in the incidence of functional disturbance between the two groups in the short-term. Furthermore, although there were significant differences in pre- and post-operative incontinence scores in both groups, there was no difference between the groups. Thus, the importance of the internal sphincter in maintenance of continence should not be underestimated. However, although this study revealed a relatively high incidence of functional disturbance, the majority of patients were satisfied with their outcome as a reasonable price to pay to be rid of chronic anal sepsis. Nevertheless, the functional consequences of surgically laying open fistulas justifies continued attempts at methods that preserve sphincter integrity and function<sup>199</sup>.

#### 1.3.4.2.4 Treatment options

The management of anal fistulas has traditionally been purely surgical. A wide range of surgical techniques have been developed, as none are universally effective at achieving the dual aims of permanent fistula eradication and the preservation of sphincteric function. At one end of the spectrum is fistula eradication, best achieved by fistulotomy, and at the other, preservation of function, with no attempt at complete eradication of the fistula, but rather palliation of symptoms, using a loose drainage seton. Various strategies have been adopted that lie between these two extremes, which can be divided between those that still divide the sphincters, but attempt to minimise the functional consequences, including the therapeutic use of setons, and those which attempt to preserve sphincteric function, such as the use of advancement flaps and more recently, modern biomaterials that can act as a scaffold for tissue repair. Unfortunately, it is virtually impossible from the literature to determine any meaningful comparisons between strategies as: patient demographics (including previous obstetric history, fistula or other anal surgery) are either inadequately reported or vary considerably; results are often not reported relative to the specific type of fistula aetiology or classification, and interpretation of fistula classification may vary (one surgeon's 'high fistula' is another's 'low fistula'); reports of success tend not to be equalled by reporting of failures or functional disturbance; and most reports contain inadequate follow-up. Additionally, in such a field of surgery, the use of prospective randomised controlled trials is difficult due to the heterogeneity of fistula anatomy, individual surgeon treatment preference, and the ethical difficulties of comparing treatment strategies in which functional outcomes may be markedly different.

Nonetheless, it is well recognised that critical to the success of all techniques in the management of anal fistulas are the elimination of acute sepsis and the eradication of any secondary fistulous extensions<sup>251</sup>. More recently, it has further been proposed that failure to adequately remove all granulation or epithelial tissue lining the fistulous tract, affects fibroblast and endothelial cell migration, and possibly in conjunction with inadequate removal of the presumed source (the diseased intersphincteric anal gland), will inhibit healing<sup>252;253</sup>.

#### 1.3.4.2.4.1 Sphincter dividing techniques

##### 1.3.4.2.4.1.1 Fistulotomy

This technique, dating back at least to the 14<sup>th</sup> century<sup>254</sup>, involves surgically dividing the tissue enclosed by the fistula tract and allowing the wound to heal by secondary intention. To date it has proven to be the most effective way of eradicating the fistula, but through division of the enclosed sphincter muscle fibres, renders the patient at risk of continence disturbance, with reported rates ranging from 5-40%<sup>255</sup>. Fistulotomy is thus usually reserved for those patients in whom the consequences of sphincter division are anticipated to result in minimal functional disturbance, for example, 'low' fistulas, traditionally interpreted as intersphincteric and transsphincteric tracks, the latter involving < 30% of the external sphincter, but not anteriorly in women<sup>256</sup>. Additionally, it is suitable for those patients, preferably with good pre-operative function and strong anal sphincters, who are prepared to risk continence to be rid of their symptoms.

Marsupialisation (suturing the divided wound edges to the edges of the fistula track) and immediate reconstruction of the divided muscle have been described in attempts

to improve wound healing and continence disturbance respectively. They have both achieved good results in comparison to conventional fistulotomy<sup>257-260</sup>. A pooling of results from studies which have compared radiofrequency compared to conventional fistulotomy have not revealed significant improvements in recurrence or incontinence scores, although there were benefits in terms of post-operative pain and healing times<sup>261-263</sup>.

#### 1.3.4.2.4.1.2 Staged fistulotomy

An alternative treatment for 'higher' fistulas is a two-stage fistulotomy, in which initially part of the sphincter beneath the primary track is divided, and a loose seton placed across the remaining sphincter. This aims to reduce the consequences of sphincter division at a single stage by allowing fibrosis in the area of division and therefore theoretically reducing retraction of that muscle divided at the second stage. At least three series have reported good fistula eradication rates using this technique, but the functional consequences (when reported) are variable, and it is difficult to draw meaningful comparisons between the studies due to the heterogeneity of the fistulas, the amount and level (proximal or distal) of sphincter divided at the first stage, the seton material employed and the varying length of follow-up<sup>264-266</sup>.

More recently, at St. Mark's Hospital the loose seton has been used with the aim of entire external sphincter preservation in transsphincteric fistulas<sup>267</sup>. The internal sphincter is divided up to the level of the internal opening (thereby removing the infecting source, the chronically inflamed anal gland in the intersphincteric space) and the external sphincter is enclosed within a loose seton. This is removed once all the wounds have healed satisfactorily (this occurs in about 60% of patients), to allow

spontaneous healing of the remaining tract. However, this technique has two drawbacks: the functional consequences of internal sphincter division; and that in a retrospective analysis covering two time periods, although there was an approximately 50% healing rate in the short term, in the longer-term, as demonstrated by a report of the same patients 8 years later, only 3 of 14 contactable patients remained healed<sup>268</sup>.

#### 1.3.4.2.4.1.3 The tight seton

The tight or cutting seton is a classic example of a traditionally termed biomaterial, and one that has been used in the surgical management of anal fistulas since the time of Hippocrates, who recorded the use of a horse hair thread, tightened intermittently 'until the enclosed flesh was eaten through'<sup>269</sup>. The rationale of the technique is similar to that of the staged fistulotomy, in that the sphincter complex is gradually severed, by repeated tightening or replacement of the seton, followed by fibrosis which supposedly prevents the divided muscle springing apart.

Table 1.7 summarises the published results of the cutting seton technique in the management of anal fistulas. Direct comparisons of the published data are difficult due to heterogeneity of fistula aetiology and anatomy, whether the first stage incorporated internal sphincterotomy, the seton material employed (Penrose drain<sup>270</sup>, stainless steel<sup>271</sup>, rubber band or elastic equivalent<sup>272-278</sup>, braided synthetic suture<sup>279;280</sup>, silk<sup>281-283</sup>, prolene<sup>284</sup> and nylon<sup>285</sup> sutures, and plastic cable ties<sup>286;287</sup>), interval and frequency of tightenings, the time taken for the seton to cut through the encircled tissue, and the varying lengths of follow-up. Nonetheless, nearly all the studies reported successful eradication of anal fistulas using this method, although



the majority had unacceptable rates of both frequency and severity of anal incontinence<sup>270;273-275;277-279;281-285;288-290</sup>.

The rates of incontinence associated with the conventional cutting seton are likely to be proportional to the speed of sphincter division. In 1986, Christensen reported a series of 24 patients with high transsphincteric fistulas in whom the seton was tightened every second day, resulting in a 62% incontinence rate, with 29% of patients requiring regular use of pads<sup>288</sup>. Ten years later, Goldberg reported a series of 13 patients, in whom the seton was tightened every second week, rather than every second day, and in that series only one patient suffered major incontinence, although 54% suffered minor incontinence<sup>274</sup>. Additionally, the technique is associated with considerable patient discomfort and there is the need for repeated replacement/ tightening of the cutting material.

There are clearly areas of modification to the cutting seton technique, which can be employed to take advantage of its excellent fistula eradication rates whilst reducing continence disturbance, the requirement for repeat tightenings and patient discomfort. These modifications to a traditionally defined biomaterial and the results of its application are described in a retrospective study in chapter 3.

**Table 1.7** Results in published studies of cutting setons for the treatment of anal fistula.

Author	Year	Study type	n	Aetiology	Anatomical Classification	Surgical IAS division	Seton Material	Time to cut through, weeks (range)	Recurrence (%)	Incontinence (%)	Duration of follow-up, months (range)
Culp <sup>270</sup>	1984	Retrospective	16	Cryptoglandular	SS	No	Elastic (Penrose drain)	Mean 2	0	Minor 15 Major 0	≥ 24
Christensen <i>et al</i> <sup>288</sup>	1986	Retrospective	21	Cryptoglandular	High TS	No	NS	Median 1 (<1-14)	0	Minor 29 Major 33	Median 96 (24-168)
Misra <i>et al</i> <sup>271</sup>	1988	Retrospective	56	Cryptoglandular	Low fistulas: IS/ low TS/ submucosal (48) High TS (8)	NS	Braided stainless steel	NS	3.5	0	NS
Ustynoski <i>et al</i> <sup>277</sup>	1990	Retrospective	11	Cryptoglandular	TS	Yes	Rubber band	Mean 7 (2-17)	18	NS	Mean 48 (8-144)
Williams <i>et al</i> <sup>278</sup>	1991	Retrospective	13	Non-Crohn's	NS	Yes	Elastic band	Median 16 (8-36)	0	Minor 54 Major 7	Median 24 (4-60)
Graf <i>et al</i> <sup>279</sup>	1995	Retrospective	29	Cryptoglandular (25) IBD (4)	High TS	Yes	Mersilene 0/0 (braided polyester)	≥ 4	8 25	Minor 44 Minor 100	Mean 46 (3-94)
McCourtney <i>et al</i> <sup>283</sup>	1996	Retrospective	27	Cryptoglandular (22) Crohn's (4) Hydradentis suppurativa, HS (1)	IS (1) TS (16) SS (5) RVF (5)	No	Silk 1/0	Median 20 (4-76)	4 (HS patient)	Minor 7 Major 4	>12
Goldberg <i>et al</i> <sup>274</sup>	1996	Retrospective	13	Cryptoglandular	TS	Yes	Rubber band	Mean 16 (8-36)	0	Minor 54 Major 7	Median 24 (4-60)

Author	Year	Study type	n	Aetiology	Anatomical Classification	Surgical IAS division	Seton Material	Time to cut through, weeks (range)	Recurrence (%)	Incontinence (%)	Duration of follow-up, months (range)
Hamalainen <i>et al</i> <sup>280</sup>	1997	Retrospective	35	NS	High TS (25) Low TS (5) SS (2) ES (3)	NS	0/0 Non-absorbable braided suture	Mean 12.5 (3-26)	6	Minor: 64 High TS 40 Low TS 100 SS 67 ES  Major NS	Mean 70 (28-184)
Dziki <i>et al</i> <sup>273</sup>	1998	Retrospective	32	NS	High TS (21) SS (4) ES (7)	Yes	Rubber band	Mean 3 (≤ 5.5months)	0	Minor: 52 High TS 100 SS & ES  Major: 9.5 High TS 50 SS 28.5 ES	Mean 16 (4-22)
Hasegawa <i>et al</i> <sup>289</sup> (abstract)	2000	Retrospective	28	Cryptoglandular	TS (>80%)	NS	NS	NS	29	Minor 25 Major 11	NS
Isbister <i>et al</i> <sup>282</sup>	2001	Retrospective	47	Cryptoglandular	High TS (16) Low TS (31)	No	Silk 1/0	Mean 24	2	Minor 36 Major 2	Mean 13
Joy <i>et al</i> <sup>275</sup>	2002	Retrospective	17	NS	TS	Yes	Snug silastic	Mean 20	6	Minor 50 (5/10)*  Major 20 (5/10)*	Mean 19 (9-54)
Durgan <i>et al</i> <sup>287</sup>	2002	Prospective case series	10	NS	ES	Yes (EAS also divided up to dentate line)	Silk 1/0	NS	0	Minor 20 Major 0	3-108

Author	Year	Study type	n	Aetiology	Anatomical Classification	Surgical IAS division	Seton Material	Time to cut through, weeks (range)	Recurrence (%)	Incontinence (%)	Duration of follow-up, months (range)
Theerapol <i>et al</i> <sup>284</sup>	2002	Prospective case series	41	NS	NS	No	Prolene 0/0	Median 9 (4-64)	2.5	0	Median 4 (<1-17)
Zbar <i>et al</i> <sup>285</sup>	2003	Prospective, randomised: Seton via IAS only <i>versus</i> Seton via IAS & EAS	34	Cryptoglandular	High TS	No	Nylon 0/0	Mean: 14 IAS only 12 IAS & EAS	11 IAS only 7 IAS & EAS	Minor: 5.5 IAS only 12.5 IAS & EAS Major 0	Median: 13 (6-30) IAS only 12 (5-28) IAS & EAS
Pescatori <i>et al</i> <sup>290</sup>	2004	Retrospective	17	NS	TS (n=?) ES (n=?)	Yes	NS	NS	NS	Minor 6 Major 18	Median 22 (5-89)
Mentes <i>et al</i> <sup>276</sup>	2004	Retrospective	20	Cryptoglandular	High TS (14) Anterior TS in females (6)	No	Elastic seton (created by cutting 2-3mm strip from surgical glove)	Mean 2.5 (1.5-4)	5	↓ baseline continence in 20% patients; no SD in pre & post op Wexner scores	6- 24
Vatasev <i>et al</i> <sup>287</sup>	2007	Retrospective	32	No Crohn's	High TS (17) SS (8) ES (7)	No	Nylon cable tie (ratcheted for tightening)	Mean 7.5 (6-10)	0	Minor 15.5 Major 0	~ 26
Gurer <i>et al</i> <sup>286</sup>	2007	Retrospective	17	Cryptoglandular	High TS (8) Low TS (4) SS (1) IS (4)	No	Nylon cable tie (ratcheted for tightening)	Mean 2.5 (1-6.5)	0	?; no SD between fistula types in post-op Wexner scores†	Mean 8 (2-15)

Author	Year	Study type	n	Aetiology	Anatomical Classification	Surgical IAS division	Seton Material	Time to cut through, weeks (range)	Recurrence (%)	Incontinence (%)	Duration of follow-up, months (range)
Chuang-Wei <i>et al</i> <sup>272</sup>	2008	Retrospective	112	No Crohn's	High TS or SS (84) ES (28)	No	Elastic band (from wrist of surgical glove)	Mean 4 (2.5-6)	1 (ES fistula)	Minor 24 Major 0	Median 38.6 (24-60)

Abbreviations: IAS, internal anal sphincter; EAS, external anal sphincter; RVF, rectovaginal fistula; TS, transsphincteric; IS, intersphincteric; SS, suprasphincteric; ES, extrasphincteric; SD, significant difference; NS, not stated.

\* Overall incontinence stated as 6% (1/17), however, continence disturbance as assessed by questionnaire (n=10) reveals tabulated result.

† No comparison between pre and postoperative continence scores performed.

#### 1.3.4.2.4.1.4 The chemical seton

An alternative to the traditional cutting seton, and one which has been used for many centuries in eastern parts of the world, is the so called chemical seton, or Kshara sutra. This seton is a thread dipped in multiple layers of agents derived from plants which, apart from endowing antibacterial and anti-inflammatory properties, impart an alkaline (approximate pH 9.5) caustic nature that essentially burns through the enclosed tissues at a rate of approximately 1cm every 6 days<sup>291;292</sup>.

Two prospective randomised trials have compared the chemical seton with fistulotomy, and shown no differences in rates of incontinence or recurrence rates, but did reveal longer healing times (8 weeks versus 4 weeks) and worse patient discomfort<sup>293;294</sup>. Furthermore, the trial designs excluded all those with anything but low transsphincteric fistulas, so it is uncertain whether such a technique has a role for the more problematic higher fistula.

#### 1.3.4.2.4.2 Sphincter preserving techniques

##### 1.3.4.2.4.2.1 Fistulectomy

Fistulectomy excises rather than incises the fistula track. However, when compared to fistulotomy, recurrences rates were similar and the time to healing was prolonged, secondary to excess tissue loss<sup>295</sup>.

The core-out technique has been advocated on the following basis<sup>296</sup>: the precise course of the track can be more accurately determined than by imaging or probing, thereby avoiding the potential creation of false tracks; core out reduces the risk of missing secondary tracks, which are seen as transected granulation tissue and thus can also be excised; the relationship of the track to the sphincter complex can be more accurately ascertained; and, a complete specimen is available for histology<sup>297</sup>.

Core out and laying open the resultant tunnel was employed in 67 patients with low fistulas, resulting in one recurrence. In 32 patients, with high transsphincteric or suprasphincteric fistulas, treated by core-out and simple stitch closure, a temporary colostomy was raised in 4 patients, and 3 had recurrences<sup>297</sup>. Unfortunately, the functional consequences were not reported in either series, and in the case of recurrent or more complex fistulas the author recommended the adoption of alternate sphincter-conserving methods.

More recently, the use of a new mechanical device, a “fistulectome”, has been described which cores-out an approximately 2mm circumferential thickness of the fistula tract<sup>298</sup>. Of 13 patients treated with this device, at a mean follow-up of 13 months, there was one recurrence and two patients developed symptoms of continence disturbance, but unfortunately the fistulas were heterogeneous in terms of aetiology and classification and it is therefore unclear in which fistulas this device may have a potential role.

#### 1.3.4.2.4.2.2 The loose drainage seton

The loose drainage seton can be used for different reasons in the management of anal fistulas<sup>248;278</sup>. A loosely tied thread can be used to drain sepsis, to allow subsidence of acute inflammation and either safer subsequent definitive surgery or as a long term palliative measure aimed at symptom control (by preventing the fistula track from occluding, and allowing sepsis to drain, thereby avoiding recurrent abscess formation). It can also be used as a marker to help determine the amount of muscle enclosed by the fistulous track, perhaps because scarring from previous surgery or relaxation under anaesthesia makes assessment difficult. In such

circumstances, the proportion of enclosed sphincter may be more accurately determined when the patient is awake and the track marked by the thread.

#### 1.3.4.2.4.2.3 Advancement flaps

The use of advancement flaps to treat anal fistulas was first documented in 1912 by Elting<sup>299</sup>. He described two key principles: separation of the track from the communication with the bowel; and adequate closure of that communication with eradication of all diseased tissue in the anorectal wall. At later dates others have added adequate flap vascularity, formation of a tension free flap, anastomosis of the flap to a site well distal to previous internal opening, and resolution of any acute sepsis prior to definitive surgery<sup>300</sup>. Most surgeons agree that the flap should include part if not all of the underlying internal sphincter in order to maintain vascularity, but even when the internal anal sphincter is preserved, despite overall resting pressures being unchanged as a group, in certain individuals there is a profound drop in resting pressure<sup>300</sup>. Additionally, advancement flaps are contraindicated in the presence of large internal openings (>2.5cm), due to the risk of anastomotic breakdown, and a heavily scarred, indurated, woody perineum precludes adequate exposure and flap mobilisation<sup>199;301</sup>.

Two literature reviews of the technique were published in 1998<sup>302</sup> and 2008<sup>263</sup>. The earlier review included all relevant publications, whereas the latter only prospective randomised controlled studies of idiopathic fistulas. However it is again difficult to compare series, as there are so many variables such as: flap thickness (mucosal, mucosal and partial internal anal sphincter, or full thickness internal anal sphincter); flap shape and orientation (caudally or cranially based); whether the internal anal sphincter is divided concomitantly; and, how the extrasphincteric component is



treated. Nevertheless, overall the reported continence and recurrence rates are extremely impressive, although interestingly when specifically compared to fistulotomy alone<sup>303</sup> or fistulotomy with sphincter reconstruction<sup>304</sup> no significant differences were reported for either outcome. Furthermore, a report by Athanasiadis<sup>305</sup>, raises an important paradigm: in a series of 224 patients, in whom internal sphincterotomy was performed (to eradicate the presumed aetiological source), the eradication rate was 79%, although this was tempered by a 19% rate of significant anal incontinence; whereas in 55 patients in whom the internal sphincter was preserved there was only a 4% rate of soiling reported, although eradication rates were not stated. Therefore, it seems that, as with all traditional surgical management strategies, the successful eradication of fistulas and the maintenance of continence remain directly competing variables.

#### 1.3.4.2.4.2.4 Use of modern biomaterials

Over the last 30 years, coloproctologists have increasingly looked towards the rapidly developing world of biologically derived materials and tissue engineering to provide a panacea for the treatment of anal fistulas: permanent fistula healing without sphincter compromise. Intuitively, the ideal biomaterial for this purpose should allow full host tissue incorporation and neovascularisation, whilst withstanding premature degradation and bacterial colonisation. To date, two novel materials have been used as part of sphincter preserving strategies for the treatment of anal fistulas: fibrin glue and lyophilised porcine-derived small intestinal submucosa (Surgisis<sup>®</sup> AFP<sup>™</sup>, anal fistula plug).

#### 1.3.4.2.4.2.4.1 Fibrin glue

Fibrin glue, the first modern biomaterial to be used in the management of anal fistulas, was initially received with great enthusiasm on account of its perceived benefits, in that the technique seemed simple to apply, repeatable (in that treatment failure did not compromise subsequent surgical options), it spared the anal sphincter mechanism, and avoided the prolonged discomfort associated with wound healing and repeated dressing changes. However, although early reports demonstrated excellent initial results, a wide-ranging variability in subsequent reports, has led to no less than six published review articles attempting to unravel the question of its uncertain efficacy<sup>263;306-310</sup>. The author of this thesis published the first of these reviews in 2004.

#### 1.3.4.2.4.2.4.1.1 Historical background

Fibrin glue (also referred to as fibrin sealant or fibrin tissue adhesive) was first used as a haemostatic agent at the beginning of the last century. During the First World War, fibrin tampons and patches were used to control bleeding from parenchymatous organs<sup>311;312</sup>. In 1944 the addition of bovine thrombin to fibrinogen allowed Cronkite *et al* to demonstrate that it could be used as a sealant to facilitate skin grafting procedures<sup>313</sup>. However, a relatively high failure rate due to poor adhesive strength and durability of the sealants meant the technique was not further pursued. In 1972, the concept of fibrin glue application in surgical procedures resurfaced<sup>314</sup>, as a method was developed which used highly concentrated fibrinogen in combination with factor XIII (fibrin stabilizing factor) and delayed fibrinolysis with aprotonin (fibrinolysis inhibitor). Further progress was made when commercial plasma fractionation methods generated concentrated fibrinogen

preparations, which were made available in Europe in the late 1970s. However, pooled fibrinogen concentrates were associated with viral transmission, leading to license revocation for fibrinogen concentrates in the United States by the Food and Drug Administration (FDA) in 1978. Since that time implementation of viral elimination procedures has abolished contamination from known viruses. Thus, in 1998, the FDA re-licensed fibrin sealant for limited operative procedures. In the interim, hospitals in the USA, on account of interest generated by the clinical success of fibrin sealants in Europe and Asia, had used as a source of human fibrinogen, autologous, single donor, or small pool cryoprecipitate fibrinogen preparations. These were mixed with bovine thrombin, providing home-made sealants, thus avoiding the risk of disease transmission<sup>307</sup>.

Fibrin glue has been used to treat a variety of fistulas, including cerebrospinal, tracheoesophageal, bronchopleural, chylous, upper gastrointestinal, pancreatic, proximal colorectal, and urological fistulas, with variable success rates<sup>307</sup>. Its use in the management of complicated perineal fistulas, albeit not specifically anal fistulas, was first reported in the early 1980s, with closure rates of 44% (9–24 months follow-up) and 80% (0–5 months follow-up)<sup>315;316</sup>.

#### 1.3.4.2.4.2.4.1.2 Mode of action<sup>307</sup>

Fibrin glue is a tissue sealant that simulates physiological clot formation. Activation is initiated by mixing a fibrinogen solution (containing fibrinogen, factor XIII, fibronectin, and aprotinin) with thrombin and calcium ions. The fibrinogen is cleaved into fibrin monomers, which loosely aggregate to form a soluble clot. Concomitantly, the thrombin and calcium ions activate factor XIII (F XIIIa), which cross links the soluble

clot into an insoluble, stable form. Fibrinolysis is retarded by the addition of a specific inhibitor, aprotinin. The F XIIIa also cross links fibronectin present in the sealant mixture, and further cross-links the fibrin and fibronectin with the collagen of the surrounding tissue. When applied to a fistula the fibrin clot: seals the fistula tract; stimulates the migration, proliferation and activation of fibroblasts; and via the bridging action of fibronectin, serves as a matrix for the in growing fibroblasts and pluripotent endothelial cells. These cells take on the function of normal tissue after fibrin degradation. Plasmin, activated from plasminogen, in the surrounding tissue causes eventual lysis of the fibrin clot and this is estimated to take 7–14 days following application. Collagen synthesis, initiated by the fibroblasts, would mark the next stage in the healing of the fistula.

#### 1.3.4.2.4.2.4.1.3 Method of instillation<sup>307</sup>

After EUA, identification of both the internal and external openings, and tract cleansing (by debridement and lavage) the glue is instilled. The individual components are mixed and warmed, then drawn up into two syringes (syringe 1: fibrinogen, factor XIII, aprotinin, and fibronectin; syringe 2: thrombin and calcium chloride solution), which are subsequently placed in a two-syringe clip, which shares a common plunger. A plastic double-lumen- Y-connector joins the two syringes. The trunk of the Y-shaped connector is then connected to a single lumen catheter, which is inserted into the tract, and if the internal opening has been left open, until the tip can be seen at the internal opening. On injection, the components mix at the tip of the catheter to form fibrin glue. Slow withdrawal of the catheter at instillation is performed and visualization of the glue should occur, if the internal opening is patent at this opening, and in all cases at the external opening. Once the glue is set the

procedure is complete; it takes 3–5 minutes for the fibrin glue to adhere firmly to the surrounding tissue and 10 min to reach 70% of its maximum strength (full strength occurs after 2 hours).

#### 1.3.4.2.4.2.4.1.4 Results of treatment

##### Fistula eradication

Fistula eradication rates have been reported to range from 0-100%, with an overall average of 50-60%<sup>307-310</sup>. This wide range of results most likely stems from differences in patient and fistula selection (in terms of aetiology and classification), treatment protocols, and follow-up duration; and as with other techniques, leads to difficulties in comparing published data.

There is little concordance in the literature as to whether healing of complex fistulas is better or worse than simple fistulas after treatment with fibrin glue. Although the one randomised controlled trial comparing fibrin glue treatment with conventional therapy (fistulotomy or loose seton insertion with or without subsequent advancement flap, depending on the assessment of complexity) concluded that although an advantage for fibrin glue was not shown for simple fistulas (fistulotomy being more successful), a statistically significant advantage for fibrin glue over conventional treatment of complex anal fistula (recurrence rates of 31% for fibrin glue versus 87% for conventional treatment) was demonstrated<sup>317</sup>.

Cited reasons for failure can be divided into those associated with recurrence after conventional therapy and those specific to fibrin glue<sup>307;318</sup>. As stated earlier, critical to the success of all techniques in the management of anal fistulas is the elimination of acute sepsis, the eradication of secondary fistulous extensions, and the adequate

removal of all granulation or epithelial tissue lining the fistulous tract<sup>318</sup>. A variety of strategies have been utilised to achieve these aims including: employing a two-stage procedure, whereby at the first procedure the fistula is simplified by extrasphincteric lay open and placement of a drainage seton, and once adequate healing has occurred, a second stage of fibrin glue instillation; electrocautery destruction of the intersphincteric anal gland; and a variety of methods to degranulate or de-epithelialise the fistula lining, mostly involving blunt curettage or abrasion with a gauze strip, although laser ablation has been described<sup>307</sup>. Certain authors have stated that the inability of fibrin glue to permanently heal anal fistulas is secondary to: a liquid consistency, allowing it to run out of the fistula tract, and that shorter tracts are therefore more prone to failure; the inability of the fibrin glue to securely close the internal opening; the potential of certain bacterial species to cause early clot degradation; and extrusion of the glue shortly after surgery because of raised intra-anal pressures. However there is insufficient evidence to determine whether shorter fistula tracts are more prone to recurrence than longer tracts, and whether measures to reduce intra-anal pressures (such as pre-operative bowel preparation, and post-operative dietary restrictions and laxatives) produce better outcomes. One randomised controlled trial has compared intra-adhesive antibiotics (100 mg of cefoxitin added to fibrin sealant), surgical closure of the internal opening (using absorbable suture), and the two strategies combined<sup>319</sup>. At one year, the final healing rates were 25%, 44% and 35% respectively but this did not reach statistical significance ( $P = 0.37$ ), and the authors concluded that these methodological adjuncts were no more successful than their historical control, treatment with fibrin sealant alone. Interestingly, a randomised study comparing internal opening advancement flap closure alone with fibrin glue instillation and flap closure resulted

in better outcomes in the former group (20% versus 46.4% recurrence,  $P < 0.05$ ), the difference attributed to the glue preventing adequate drainage deep to the flap<sup>320</sup>; these findings are further supported by two subsequent case-series<sup>321;322</sup>.

There are also biological factors, specific to fibrin glue, that are likely to account for its failure to permanently heal anal fistulas, and these are two-fold. Buchanan *et al* demonstrated that epithelialisation over the external opening of the fistula tract is often misinterpreted as evidence of fistula healing, and in such circumstances recurrence is inevitable<sup>252</sup>. Indeed, studies have shown that although fibrin glue encourages fibroblast migration and epithelialization across its surface, it does not permit fibroblastic infiltration or the synthesis of crucial extracellular matrix proteins<sup>323;324</sup>. Additionally, the rate of fibrin glue degradation may limit its ability to act as a scaffold for tissue repair, and studies have shown that the majority is resorbed within five to ten days, which is insufficient time for establishment of a permanent extracellular matrix<sup>253;325</sup>.

#### Continence disturbance

All the reports in the literature comment on the maintenance of continence after the instillation of fibrin glue. However, only 3 studies have specifically assessed continence<sup>317;326;327</sup>. El-Shobaky *et al* compared 30 anal fistulas treated with fibrin glue to 30 matched anal fistulas treated by fistulotomy<sup>326</sup>. Those treated with fibrin glue were noted to have no impairment of postoperative anal sphincter function opposed to those treated by fistulotomy, in whom one patient remained incontinent for flatus and two patients had minimal soiling which persisted for more than 3 months. Zmora *et al* retrospectively noted any recorded symptoms of incontinence in

the pre- and postoperative medical records of 37 patients treated with fibrin glue and rectal advancement flap, and contacted patients by telephone to assess long-term outcome and anal sphincter function. They were no symptoms of incontinence noted in the patients' medical records and none of the patients reported any change in anal continence<sup>327</sup>. Lastly, in the only randomised comparative trial of fibrin glue versus conventional treatment for anal fistula, no change in either continence scores or sphincter pressures were reported in those patients treated with fibrin glue compared to 15% of patients managed with traditional surgical techniques<sup>317</sup>.

#### Other complications

Septic complications, including abscess formation and the development of further secondary extensions, have been reported in up to 10% of cases<sup>317;326;328;329</sup>, and rare cases of allergic reaction to fibrin glue have been reported<sup>307</sup>



#### 1.3.4.2.4.2.4.2 Anal fistula plug (Surgisis<sup>®</sup> AFP<sup>™</sup>)

The anal fistula plug (AFP) is composed of lyophilised porcine-derived small intestinal submucosa. To date, Surgisis<sup>®</sup> has been used to treat enterocutaneous fistulas; incisional, inguinal and para-oesophageal hernias; and, as a urethral sling in urogynaecological procedures<sup>77;169;170;330-332</sup>. The biological properties of this material, and its potential to act as bioscaffold for soft tissue repair and reinforcement, have been discussed in the introductory section on the role of SIS in parastomal hernias. In 2006, the first report of the use of Surgisis<sup>®</sup> to treat anal fistulas, as a biological plug, was published<sup>333</sup>. The authors cited the reasons for employing the plug were that it overcame the technical failures of fibrin glue as previously described, and due to the relative success of Surgisis<sup>®</sup> in treating hernias in potentially contaminated and contaminated wounds. Interestingly, as discussed in the previous section on fibrin glue, those studies which employed measures to counteract the former concerns (for example, occlusion of the internal opening to prevent intra-operative leakage of fibrin glue, and bowel preparation to avoid early bowel movements, and consequent early clot extrusion), do not report better success rates than those that did not use either protocol<sup>318</sup>. Additionally, those studies that employed Surgisis<sup>®</sup> to reconstruct abdominal wall defects in contaminated fields, reported accelerated degradation of the implant, which the authors hypothesized was responsible for early hernia recurrence<sup>77;170</sup>.

#### 1.3.4.2.4.2.4.2.1 AFP procedure

In 2007, on account of concerns of lower success rates with this procedure than those initially published (83% at 12 months follow up<sup>333;334</sup>), surgeons experienced with the AFP convened to develop a consensus paper on the proper technique,

patient selection criteria, and pre- and postoperative management in order to maximise its success<sup>335,336</sup>. The procedure is indicated in all Park's classification of fistulas, including those of cryptoglandular and Crohn's aetiology, which are unsuitable for fistulotomy (due to the amount of sphincter involved or poor pre-operative function). It is contraindicated in those with pouch or rectal vaginal fistulas, acute sepsis (which if identified, placement of a drainage seton is suggested) but not secondary extensions, patients with an allergy to porcine products, and an inability of the surgeon to identify both the internal and external openings. No recommendations are made regarding bowel preparation, although a single dose of preoperative systemic antibiotics is advised. After EUA and identification of both the internal and external openings, the tract should be irrigated with either saline or H<sub>2</sub>O<sub>2</sub>. Debridement, curettage or brushing is not advised, since this may lead to a larger tract and risk expulsion of the plug. A suture or ligature is placed at the narrow end of the plug and then pulled from the internal to the external opening until the plug is snug. Excess plug should be trimmed from the internal opening, and an absorbable suture placed, incorporating the internal sphincter, to close the os and anchor the plug. The excess external plug is then excised flush with the skin, and the external opening left open to allow drainage of any exudate. Post-operatively, no restriction in diet is recommended, but constipation and diarrhoea should be prevented or treated. Patients are advised to refrain from strenuous or sexual activity, and heavy lifting for 2 weeks, in order to avoid dislodgment of the plug. During follow-up visits the tract should not be probed.

#### 1.3.4.2.4.2.4.2.2 Results of treatment

Since the first report of this technique there have been at least 11 studies, published in full text, on the AFP; these are summarised in Table 1.8. The majority (8 of 11) are prospective case-series, and the remainder comprise retrospective studies.

#### Fistula eradication in idiopathic fistulas

Fistula eradication rates range from 24 - 93%, with an overall average of 50 - 60%<sup>322;334;337-345</sup>. As with fibrin glue and other fistula treatment strategies, differences in patient demographics (such as previous fistula surgery), fistula aetiology and classification, treatment protocols, duration of follow-up, small participant numbers, and the absence of randomised controlled trials, mean comparisons between published data are almost impossible. However, two non-randomised studies have compared the AFP technique with other sphincter preserving procedures: Johnson *et al* compared the short-term results of two prospective groups of patients with high transsphincteric fistulas, and using fibrin glue in the alternate study arm demonstrated a significantly higher rate of fistula eradication for those treated with the AFP (87% vs 40%,  $P < 0.05$ )<sup>333</sup>; Ellis compared retrospectively collected data on patients treated with mucosal advancement flap repair or the AFP, and despite a trend in favour of the AFP showed no significant difference in overall fistula eradication between the two groups (67% vs 88% respectively, P value not published)<sup>339</sup>. The results of a randomised controlled multi-centre trial, comparing mucosal advancement flaps with the AFP for the treatment of cryptoglandular high transsphincteric fistulas, are awaited<sup>346</sup>.

The reported fistula eradication rates incorporate single tract fistulas, and those with secondary extensions and horse-shoe configurations. Despite the widely accepted view that untreated secondary and horseshoe extensions are significantly associated with fistula persistence<sup>251</sup>, this stance is not upheld in those studies that addressed this issue in the setting of the AFP technique<sup>334;340;341;344</sup>. It could be argued however that the numbers involved in this subset of patients are too small to draw any meaningful conclusions. From the data available, those variables which have been shown to significantly affect closure rates include height of the fistula (the higher the fistula, the lower the chance of fistula eradication,  $P < 0.05$ )<sup>337</sup>, and whether the fistula was undergoing plug placement for the first-time or a repeat procedure (first vs repeat attempt: 64% vs 12.5%,  $P = 0.011$ )<sup>341</sup>. The authors of the former observation further demonstrated that the higher failure rate in higher fistulas (and therefore presumably those with longer tracts), was at least partly secondary to higher plug extrusion rates (< 1/3 vs 1/3 - 2/3 vs >2/3 EAS involvement: 7% vs 19% vs 38% plug extrusion respectively,  $P = 0.04$ ). These findings are in conflict with the anecdotal reports from other surgeons, recognised as experienced in the plug technique, who suggest that plug extrusion is more likely in short tracts, thus the contraindication for AFP use in rectovaginal fistulas<sup>335;336</sup>. Nonetheless plug extrusion is a recognised cause of plug failure, and has been reported to occur in 10-41% of patients<sup>336;337;343-345</sup>. This is despite the majority of studies performing pre-operative bowel preparation and/ or prescribing post-operative dietary restrictions and laxatives, in order to avoid raised intra-anal pressures (the presumed cause of early fibrin glue expulsion). Other reasons cited for early plug extrusion include the tract being too wide, the plug being pulled too tightly, and inadequate plug fixation<sup>336</sup>.

Two studies deviated from the recommended operative technique by using the AFP in conjunction with a dermal or mucosal advancement flap, and both reported eradication rates of 67%<sup>341;342</sup>. However, as there were no control groups, no benefit over the standard AFP protocol can be demonstrated.

**Table 1.8** Results of published studies (in full text) of the anal fistula plug (AFP)

Author	Year	Study type	N	Aetiology	Anatomical Classification	Management Protocol*	Fistula eradication (%)	Incontinence (%)	Other Complications	Duration of follow-up, months (range)
Champagne <i>et al</i> <sup>334,xx</sup>	2006	Prospective case-series	46	Cryptoglandular	High TS	<ul style="list-style-type: none"> <li>• Bowel preparation</li> <li>• Abx prophylaxis</li> <li>• Post-op: Liquid diet for 48hrs/ topical flagyl/ activity restriction</li> </ul>	38/46 (83%) <sup>‡</sup>	NR	NR	Median 12 (6-24)
O'Connor <i>et al</i> <sup>347</sup>	2006	Prospective case-series	20	Crohn's	-	As above	16/20 (80%)	NR	NR	Median 10 (3-24)
Ellis <sup>339</sup>	2007	Retrospective	18	13 Cryptoglandular	TS	<ul style="list-style-type: none"> <li>• Nil pre-op protocol</li> <li>• Post-op: Laxatives/ activity restriction</li> </ul>	12/ 13 (92.5%)	NR	NR	Median 6 (3-11)
				5 Crohn's	RVF		4/5 (80%)			
van Koperen <i>et al</i> <sup>345</sup>	2007	Prospective case-series	17	14 Cryptoglandular	High TS	<ul style="list-style-type: none"> <li>• Bowel preparation</li> <li>• Abx prophylaxis</li> <li>• Post-op: Activity restriction</li> </ul>	4/14 (28.5%)	NR	NR	Median 7 (3-9)
				1 Crohn's	-		1/1 (100%)			
				2 HIV	-		2/2 (100%)			

Author	Year	Study type	N	Aetiology	Anatomical Classification	Management Protocol	Fistula eradication (%)	Incontinence (%)	Other complications	Duration of follow-up, months (range)
Christoforidis <i>et al</i> <sup>337</sup>	2008	Retrospective	46	39 Cryptoglandular 3 Crohn's 3 Post-IPAA/ IAA	39 TS 4 SS 2 IS 2 Anovaginal	<ul style="list-style-type: none"> <li>Bowel preparation</li> <li>Post-op: Nil protocol</li> </ul>	20/47 (42.5%) <sup>†</sup> (31% Cryptoglandular)	NR	2 acute post-op sepsis 9 c/o ↑ fistula discharge	Median 5 (1-11)
Schwandner <i>et al</i> <sup>343</sup>	2008	Prospective case-series	18	11 Cryptoglandular 7 Crohn's	19 TS	<ul style="list-style-type: none"> <li>Bowel preparation</li> <li>Abx prophylaxis</li> <li>Post-op: Activity restriction</li> </ul>	5/11 (45.5%) 6/7 (85.7%)	No change in Cleveland or QoL scores (0%)	NR	Mean 10 (SD 2.5)
Lawes <i>et al</i> <sup>342</sup>	2008	Retrospective	17 AFP alone 3 AFP & transanal flap	20 Cryptoglandular	17 TS 3 Ano-perineal	<ul style="list-style-type: none"> <li>Abx prophylaxis</li> </ul>	4/17 (24%) 2/3 (67%)	NR	5/17 (29%) acute post-op sepsis	Mean 7.4
Thekkinkattil <i>et al</i> <sup>344</sup>	2008	Prospective case-series	43	32 Cryptoglandular 7 IBD 3 IPAA 1 Other	29/32 TS 3/32 SS	<ul style="list-style-type: none"> <li>Bowel preparation</li> <li>Post-op: Oral abx/ laxatives/ activity restriction</li> </ul>	17/32 (53%) <sup>‡</sup> (55%, TS; 0% SS) 3/11 (27%)	NR	NR	Median 10.5 (3-17)

Author	Year	Study type	N	Aetiology	Anatomical Classification	Management Protocol	Fistula eradication (%)	Incontinence (%)	Other complications	Duration of follow-up, months (range)
Ky <i>et al</i> <sup>341</sup>	2008	Prospective case-series	44 AFP & mucosal flap	30 Cryptoglandular (4 RVF; 1 horseshoe)  14 Crohn's	-	<ul style="list-style-type: none"> <li>• Bowel preparation</li> <li>• Abx prophylaxis</li> <li>• Post-op: Topical flagyl/ oral antibiotics/ laxatives/ activity restriction.</li> </ul>	20/30 (67%) <sup>‡</sup>  4/14 (28.5%) <sup>*‡</sup>	0%	5/44 (11%) acute post-op sepsis	Median 6.5 (3-13)
Echenique <i>et al</i> <sup>338</sup>	2008	Prospective case-series	23	Cryptoglandular	-	-	14/23 (61%)	NR	3/20 (15%) acute post-op sepsis	-
Garg <sup>340</sup>	2008	Prospective case-series	21	Cryptoglandular	High TS	<ul style="list-style-type: none"> <li>* Bowel preparation</li> <li>* Abx prophylaxis</li> <li>* Post-op: Liquid diet for 48 hrs/ Topical flagyl/ oral antibiotics/ laxatives/ activity restriction</li> </ul>	15/21 (71.5%) <sup>‡</sup>	0%	1/21 (5%) acute post-op sepsis	Mean 9.5 (6.5-18)



Abbreviations: RVF, rectovaginal fistula; TS, transsphincteric; IS, intersphincteric; SS, suprasphincteric; Abx, antibiotics; NR, not reported; IBD, inflammatory bowel disease; IPAA, ileal pouch anal anastomoses; IAA, ileo-anal anastomosis.

\* All studies report uniformity in tract management (no curettage, only saline or H<sub>2</sub>O<sub>2</sub> lavage), plug placement, suture closure of internal os, and leaving the external os open for drainage.

\*\* This study presents the longer term results of a previously published study by Johnson *et al*<sup>33</sup>, hence only the later paper is included.

† The only significant variable was the height of the fistula; the higher the fistula the lower the chance of fistula eradication.

‡ No significant difference in closure rates between those fistulas with single vs multiple tracts or horse-shoe configurations.

¥ Significant difference ( $p \leq 0.05$ ) in closure rates between Crohn's and Non-Crohn's fistulas

## Continence and other complications

Only 3 publications report on continence as an outcome measure. Schwander *et al* focussed on functional outcome and quality of life, using the Cleveland Clinic Florida Incontinence Score (CCFIS) and the Faecal Incontinence Quality of Life Scale (FIQL). They documented no difference in the pre-and post-operative CCFIS, and significant improvements in the FIQL depression/ self-perception and embarrassment scales ( $P<0.01$ )<sup>343</sup>. Ky *et al* simply stated that no incidence of incontinence to stool or flatus was found at post-operative interviews<sup>341</sup>, and Garg reported that post procedure none of the patients complained of any change in continence<sup>340</sup>.

Septic complications, including abscess formation and worsening of fistula discharge, have been reported in a range of 5 – 29% of cases, with an overall mean incidence of 17%<sup>337;338;340-342</sup>.

### 1.3.5 Conclusion

Although the successful management of anal fistulas has traditionally been surgical, the functional risks to the anal sphincter complex mean fistula surgeons will continue to explore new methods that avoid such compromise, and the variable success of biological agents, such as fibrin glue and the anal fistula plug, show that they have potential to achieve these hitherto conflicting outcomes. Permacol<sup>®</sup> is a porcine-derived dermal collagen implant which is cross-linked to impede enzymatic degradation. As with Surgisis<sup>®</sup>, its biological properties have been discussed in the section on parastomal hernias. The potential role of this cross-linked collagen

implant in the management of idiopathic anal fistulas is investigated in a prospective phase 1 study presented in chapter 4.

# Chapter 2

Parastomal Hernia Prevention Using A Novel Collagen  
Implant: A randomised controlled clinical (phase 1) and  
histological study

## 2.1 Introduction

Parastomal hernias have a reported incidence of up to 70%, increasing with the length of follow-up<sup>33;35;41;42</sup>. They can lead to complications ranging from poor cosmesis, mild discomfort and difficulty with appliance application (causing skin irritation and leakage of bowel contents) to life-threatening complications such as strangulation, obstruction and perforation<sup>31;36-38</sup>. Up to 70% of patients require surgical repair<sup>33;35</sup>, and, of the documented techniques, prosthetic mesh repair (to reinforce the edges of the stoma trephine) is the most efficacious, although it still has reported recurrence rates of up to 8%, as well as the associated morbidity and cost of a second procedure<sup>33</sup>. As such, certain surgeons have recognised that prevention of parastomal hernias may be the best approach<sup>33;56</sup>. To date, a number of studies have reported encouraging results regarding the prophylactic placement of polypropylene mesh in an attempt to reduce the rate of parastomal herniation<sup>98;123;125-127</sup>.

Although only polypropylene (and modifications thereof), have been described to prevent parastomal hernias, several different synthetic and biologically derived materials have been used to repair parastomal hernias (see Chapter 1). This is predominantly as none completely succeed in fulfilling the hernia surgeons' requirements of the ideal abdominal wall repair material, which include adequate strength for the intended surgical application, surgeon friendly handling characteristics, and promotion of host tissue in-growth. Such a material must also fail to elicit an acute hypersensitivity reaction or rejection, or to induce a chronic inflammatory or foreign body reaction (biocompatibility), and be capable of sterilization<sup>130;131</sup>.

Traditionally, non-absorbable synthetic materials, such as polypropylene, have been employed as they are associated with the lowest rates of hernia recurrence<sup>164;348</sup>. However, polypropylene mesh strengthens the abdominal wall both by mechanical tension and by induction of a strong chronic inflammatory foreign body response<sup>144</sup>. This consequently results in mesh contraction and formation of an avascular fibrotic conglomerate<sup>146</sup>, with the potential for bowel fistulation, erosion into abdominal viscera, intraperitoneal adhesions, and increased susceptibility to infection<sup>169;349-351</sup>. Furthermore, if complications occur, mesh extraction can be challenging due to dense tissue incorporation.

Absorbable repair materials have also been used, and have the advantage of an improved host tissue response. These include synthetic materials, such as polyglactin, and xenografts, such as ovine and porcine dermal collagen and bovine pericardium<sup>158;159;161</sup>. However they are not indicated when prolonged tensile strength is required<sup>160;352</sup>, as their use is associated with frequent reherniation rates as a consequence of premature implant degradation before adequate tissue in-growth has occurred<sup>161</sup>. Use of chemically cross-linked xenogeneic implants, with the aim of impeding the rate of resorption, has significantly decreased the reherniation rate<sup>159;161;162;182;183;191</sup>.

An acellular cross-linked collagen sheet derived from porcine dermis (Permacol<sup>®</sup>, Tissue Science Laboratories, Aldershot, UK) has been used successfully for laparoscopic inguinal and parastomal hernia repair<sup>79;353</sup>, repair of large abdominal wall defects<sup>191;194</sup>, and general surgical soft tissue augmentation in both animals and humans<sup>182;184-186</sup>. Comparative studies with polypropylene in rat models have demonstrated that it has better tissue compatibility, with less adhesion formation,

more orderly collagen deposition and comparable tensile strength at 90 days after implantation<sup>183</sup>.

The aim of this phase 1 study was to assess the safety, feasibility, and potential efficacy of preventing parastomal hernias using this cross-linked collagen implant. Additionally, biopsies of the collagen implant were obtained from patients who have since undergone stoma reversal, providing a unique opportunity to evaluate its biocompatibility, degradation, and cell integration. Host neo-extracellular matrix (ECM) protein deposition and neovascularisation were also evaluated.

## **2.2 Materials and methods**

The study was approved by the local ethics committee (REC reference: P/02/263).

### **2.2.1 Patients**

All patients requiring a defunctioning loop ileostomy, performed as part of an elective procedure, were prospectively invited to participate in the study on an intention-to-treat basis. After obtaining informed consent, patients were randomised, by means of opening consecutively numbered sealed envelopes, to receiving either a conventional loop stoma or the same procedure with addition of the collagen implant. Patients were blinded as to which arm of the trial they had been entered. Patient age, sex, and body mass index (BMI) were recorded. Details on previous abdominal surgery and the primary procedure requiring a loop stoma were also recorded.

### **2.2.2 Materials**

Permacol<sup>®</sup> is a porcine-derived acellular dermal sheet, predominately composed of type I collagen (93–95 per cent), with type III collagen and a small amount of elastin

comprising the remainder. Its manufacture involves trypsinization (to remove all living cells and non-collagenous debris), solvent extraction (to remove all lipid and fat deposits),  $\gamma$  irradiation and cross-linkage with hexamethylene-diisocyanate<sup>186</sup>. The implant contains naturally occurring pores, in the form of hair follicle remnants, which number 5–13 pores/cm<sup>2</sup> and are 254–654  $\mu$ m in diameter. Sterile sheets 10 × 10 cm in size and 1.0 mm thick were used, which were kept moist in sterile saline. The sheets were double vacuum packed and heat sealed in sachets of aluminium foil (inner) and polyester/polythene (outer), and stored at room temperature.

### **2.2.3 Surgical technique**

#### **2.2.3.1 Stoma formation**

All patients had a circular incision (approximately 2cm in diameter) at a pre-marked skin site, followed by a 2 x 2 cm cruciate incision in the anterior rectus sheath, and where present (ie. above the arcuate line) in the posterior rectus sheath. A trephine was subsequently created through all the layers of the anterior abdominal wall. In those receiving the implant, initially the potential space between the subcutaneous fat and the anterior layer of the rectus sheath (the fascial onlay position) was dissected in all directions around the trephine to allow the subsequent placement of the Permacol<sup>®</sup> implant (five patients), although this was later changed (for reasons discussed later) to between the posterior layer of the rectus sheath and the peritoneal membrane (preperitoneal position; ten patients).

A cylindrical defect, approximately 2 cm in diameter, was fashioned in the centre of the collagen sheet, and the implant was inserted into the previously created plane



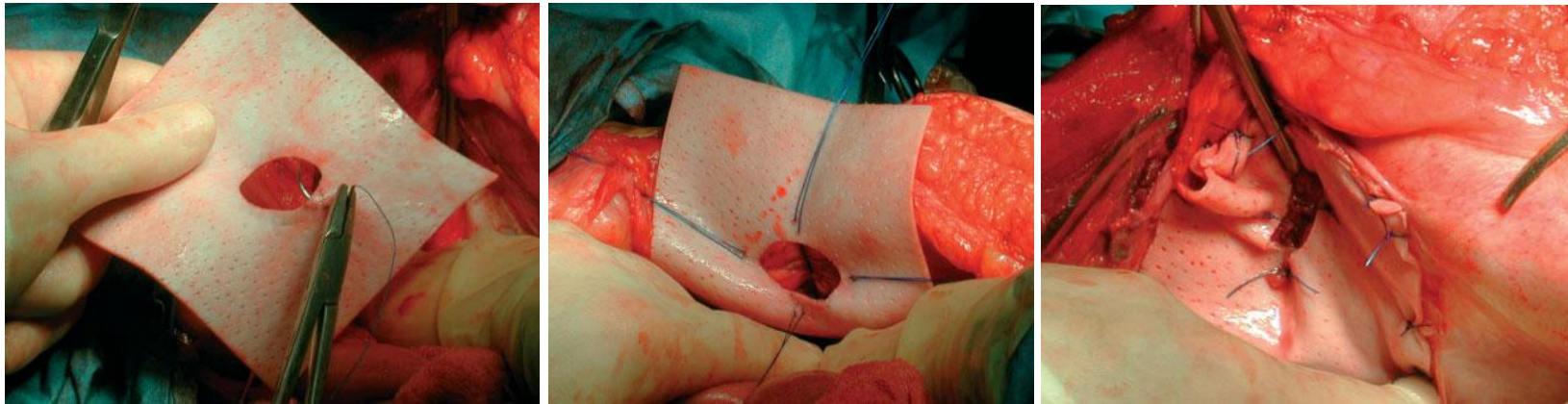
(Figure 2.1(A)). The central defect was sutured to the appropriate layer of the rectus sheath (at the 12, 3, 6 and 9 o'clock positions), using interrupted 3/0 prolene sutures, so as to encircle the abdominal trephine (Figure 2.1 (B)). The outer four corners of the implant were also sutured to the rectus sheath in the same fashion (Figure 2.1 (C)). The cut edge of the peritoneum was sutured to the corresponding edge of the posterior layer of the rectus sheath to enclose the implant.

In all patients, the appropriate loop of bowel was brought through the peritoneum, the implant (if present), and the remaining layers of the anterior abdominal wall, without any tension. The stoma was fashioned in the standard manner using 3/0 vicryl rapide<sup>®</sup>.

#### **2.2.3.2 Stoma reversal**

In those undergoing stoma reversal, the bowel was dissected down to the peritoneal cavity. If present, the collagen implant was biopsied, and the opening in the bowel either primarily closed with 3/0 vicryl or the adjacent bowel resected and anastomosed using a linear stapler. The peritoneum, rectus sheath and, if present, the implant trephine, were closed using either 1/0 loop PDS or interrupted 1/0 nylon sutures. The skin was closed in the standard manner using staples.

Biopsy specimens were taken from the edge of the implant trephine, and immediately fixed in 4% formal saline.



A. Implant before insertion

B. Sutured central trephine

C. Sutured outer four corners

**Figure 2.1.** (A) Implant with a central trephine measuring 2 cm in diameter, just before insertion within the preperitoneal space. (B) Central trephine of implant sutured to the posterior layer of the rectus sheath (at 12, 3, 6 and 9 o'clock positions), using interrupted polypropylene sutures, so as to encircle the abdominal trephine. (C) Outer four corners of the implant sutured to the rectus sheath in the same fashion as the edges of the central trephine.

#### 2.2.4 Follow up

Patients were followed-up until the time of stoma reversal or, in the event of the stoma not being reversed, until 12 months after stoma formation. Patients completed a questionnaire assessing for symptoms associated with parastomal herniation on a monthly basis, and underwent a clinical examination for signs of a parastomal hernia, and other complications, at 6 weeks postoperatively and then every 3 months until stoma reversal or 12-months post-stoma formation. In those patients whose stomas were reversed, at the time of the second procedure any evidence of stomal herniation was recorded. Serum white cell count, C-reactive protein levels and erythrocyte sedimentation rates were performed on a monthly basis, for 6 months, to establish whether there was any serological evidence of a systemic inflammatory response related to the presence of the implant. Ultrasound examination of the stoma site was performed at least 3 months after stoma formation, usually on the day prior to reversal, to detect for evidence of localised chronic seroma formation related to the presence of the implant.

#### 2.2.5 Histological and immunohistochemical examination

After fixation, appropriate samples were embedded in paraffin and 5- $\mu$ m thick sections were cut. Samples for histology were stained with haematoxylin and eosin and Masson's trichrome. The latter stains the nuclei of the cells blue-black, cytoplasm, muscle and erythrocytes stain red, and collagen stains blue<sup>354</sup>. Samples for immunohistochemistry were incubated with the antibodies of interest. The avidin biotin complex method was used. The antibodies used to determine the nature of the host inflammatory response, and those to identify specific ECM protein deposition and neovascularization, are summarised in Table 2.1. The presence of the various

inflammatory cells was quantified microscopically. Five fields per slide were counted at a magnification of x40 (Leica DMR; Leica, Solms, Germany) by two independent observers; three slides per patient were analysed. These fields were selected randomly within the collagen implant itself, at the native pores within the implant, and at the interface between the implant and surrounding host tissue. For descriptive purposes, a histological scoring criterion analogous to that described previously was used<sup>183;355</sup>: cellular presence was ranked as absent (no cells/field), mild (1–5 cells/field), moderate (6–10 cells/field) or severe (more than 10 cells/field). Evidence of neo-ECM protein deposition, neovascularization and their patterns of distribution were described qualitatively. Neovascularization was defined by the presence of structures exhibiting typical vascular walls and staining positively for laminin. The organization and composition of host neocollagen was determined by examination of Masson's trichrome-stained sections. The implant could be distinguished clearly from human tissue by its distinct morphological appearance.

### **2.2.6 Statistical analysis**

Comparisons were made between the two groups, stoma reinforcement with mesh and conventional stoma (Fishers exact test). Analysis was performed using a commercially available software package (Prism 4, Graftpad software, San Diego, CA, USA). Statistical significance was assigned at the 5% level.

Formal statistical analysis was not performed on the histological data, as this was primarily assessed in a descriptive manner. However, summary data have been provided as median (range) values (Table 2.4).

**Table 2.1** Antibodies used for immunochemistry

<b>Antigen</b>	<b>Cell type/ process identified</b>	<b>Dilution</b>	<b>Supplier</b>
CD3	T cells	1:250	Labvision
CD4	T-helper cells	1:100	Dako
CD8	T-suppressor cells	1:50	Dako
CD20	B cells	1:400	Dako
CD57	Natural killer cells	1:30	Novocastra
CD68	Macrophages	1:4000	Dako
CD138	Plasma cells	1:100	Dako
Myeloperoxidase	Granulocytes	1:2000	Dako
Vimentin	Fibroblasts	1:8000	Dako
Fibronectin	ECM protein deposition	1:1000	Novocastra
MMP-1	ECM protein deposition	1:100	Novocastra
Laminin	Neovascularisation	1:500	Novocastra

MMP, matrix metalloproteases; ECM, extracellular matrix. Labvision, Thermofisher Scientific Runcorn, UK; Dako, Ely, UK; Novocastra, Vision BioSystems, Newcastle upon Tyne, UK.

## 2.3 Results

Twenty-five patients were included in the study. Fifteen were randomised to receiving the mesh, and ten to a conventional stoma. Patient demographic and relevant surgical data are summarised in Table 2.2.

**Table 2.2** Patient and operative characteristics

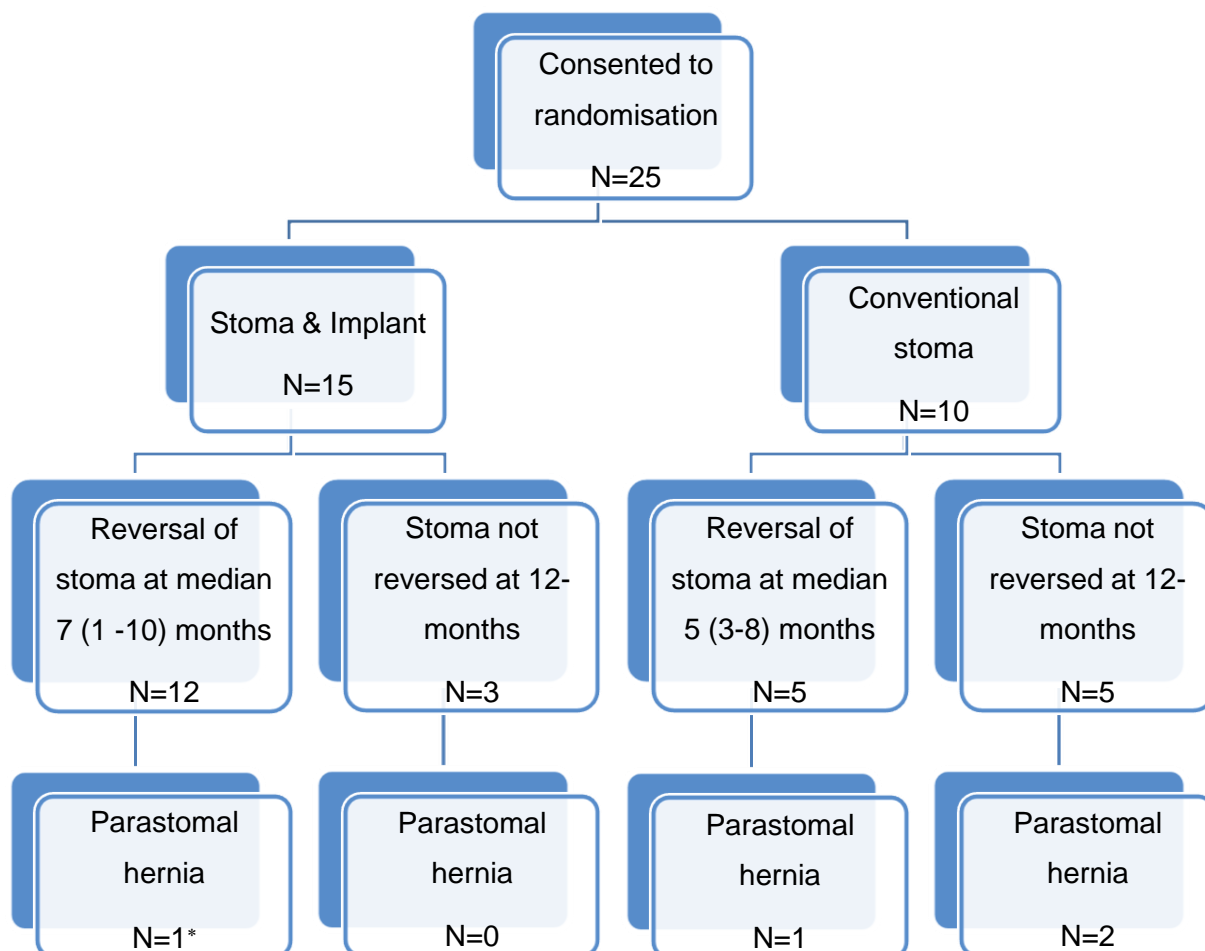
<b>Demographics</b>	<b>Conventional stoma (N=10)</b>	<b>Stoma + implant (N=15)</b>
Median age in years (range)	50 (22-70)	43 (21-69)
Sex ratio (M:F)	4 : 6	6 : 9
Median BMI (range)	26.3 (20.1 – 44)	27 (22.6 – 31)
Median no. of previous abdominal operations (range)	1 (0 - 2)	1 (0 – 3)
<b>Indication/1° procedure</b>		
<i>Megarectum/rectal reduction</i>	1	1
<i>Slow transit constipation/loop ileostomy</i>	1	1
<i>Faecal incontinence/gracilis neosphincter</i>	3	5
<i>Rectal cancer/anterior resection</i>	3	4
<i>Ulcerative colitis/proctocolectomy and pouch</i>	2	4

### 2.3.1 Clinical and operative

The clinical and operative findings are summarised in a flow chart (Figure 2.2). Of the ten patients randomised to receiving a conventional stoma, 5 had their stomas reversed at a median of 5 (range 3–8) months, and 3 patients had evidence of parastomal herniation. Of the 15 patients recruited to receiving the implant, 12 underwent stoma reversal at a median of 7 (range 1–10) months. The first 5 patients recruited to this arm of the trial had the implant sited in the fascial onlay position, of which one developed a hernia between the implant and the anterior layer of the rectus sheath, prompting subsequent preperitoneal placement of the implant in the following 10 patients. None of these ten patients had any evidence of parastomal herniation. There was no significant difference between the two arms ( $P=0.31$ ).

At the time of stoma reversal, the collagen implant was found to be present and intact in 11 of the 12 patients. The peritoneal and muscular surfaces of the implant had become bordered with non-fibrous, well-vascularised connective tissue with mild-to-moderate adherence. Fibrous scar tissue was only evident at the suture sites. Adherence to bowel serosa was absent or minimal. The presence of the implant did not complicate reversal of the stoma. The remaining patient developed a multi-resistant *Staphylococcus aureus* wound infection after stoma formation, which presumably resulted in the implant being fully degraded, as there was no evidence of the implant at the time of re-operation.

There were no other infective complications, and no patient experienced fistula formation or bowel erosion. Reasons for patients not undergoing stoma reversal by 12 months included patient preference ( $n = 3$ ), patient co-morbidities preventing further complex surgery ( $n = 2$ ), recurrent anal carcinoma requiring proctectomy ( $n = 1$ ), severe pouchitis ( $n = 1$ ), and prolonged chemotherapy for advanced rectal cancer ( $n = 1$ ).



**Figure 2.2** Flow chart of clinical and operative findings.

- \* This patient had the implant sited in the fascial onlay position, and the hernia developed between the implant and the anterior layer of the rectus sheath, prompting pre-peritoneal mesh placement in subsequent patients.



### 2.3.2 Patient questionnaire

The results of the patient questionnaire are summarised in Table 2.3. Of the ten patients randomised to receiving a conventional stoma, three documented the presence of a parastomal bulge, which corresponded to the same three patients in whom a clinically detected parastomal hernia was evident. These three patients documented symptoms related to the presence of a parastomal hernia, including difficulty with bag application, leakage of stoma bag contents, nausea, vomiting, bloating, and parastomal discomfort. Of the fifteen patients recruited to receiving the implant, two documented a parastomal bulge, one of whom was the patient who had developed a hernia between the anterior rectus sheath and the implant. This patient's predominant symptom was difficulty with bag application and discomfort. The other patient had no hernia evident on clinical examination, ultrasound examination or at the time of stoma reversal. One patient complained of symptoms of intermittent small bowel obstruction (nausea, vomiting, bloating and cessation of wind and stool per stoma) shortly after stoma formation, prompting early stoma reversal (1 month post initial stoma formation); no hernia was detected clinically, on ultrasound or at the time of stoma reversal, but a loop of small bowel proximal to the stoma was found to be wrapped around an intra-peritoneal adhesive band at a distance from the abdominal wall trephine and implant. Another patient (1 of 15) complained of regular nausea, vomiting and bloating but these symptoms were unaltered in frequency or severity compared to those experienced pre-stoma formation.

**Table 2.3** Results of patient questionnaire

Symptoms	Conventional stoma	Stoma + implant
	N=10	N=15
Stomal bulge	3	2 <sup>A, B</sup>
Bulge → difficult bag application	2	1 <sup>B</sup>
Stoma bag leakage	2	0
Bulge → pain	1	1 <sup>A</sup>
Stoma ceases to produce flatus	0	1 <sup>C</sup>
Stoma ceases to produce stool	0	1 <sup>C</sup>
Nausea & vomiting	2	2 <sup>C, D</sup>
Abdominal bloating	2	2 <sup>C, D</sup>

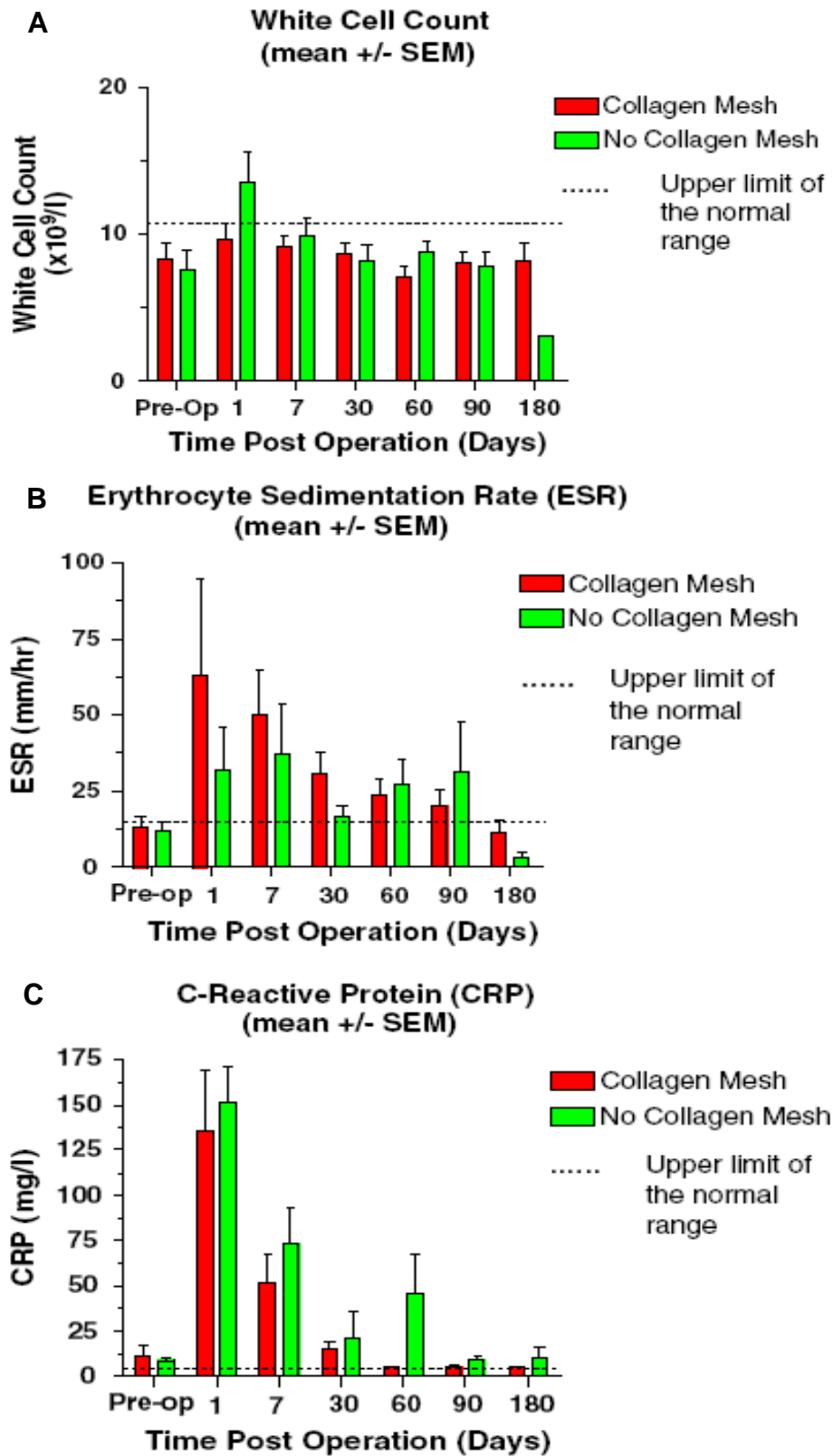
(A), patient in whom hernia developed between anterior layer of rectus sheath and fascial onlay implant; (B), No hernia evident on clinical examination, ultrasound or at time of stoma reversal; (C) Symptoms secondary to small bowel obstruction secondary to intra-peritoneal adhesions; (D), Symptoms present pre-operatively and no hernia evident on clinical examination, ultrasound or at time of stoma reversal.

### 2.3.3 Stoma site ultrasonography

Of the 10 patients randomised to receiving a conventional stoma, nine of ten underwent stoma site ultrasonography at a median of 5 (range 3–12) months post-operation, and none (0 of 9) had ultrasonographic evidence of a chronic seroma or fluid collection. Of the 15 patients randomised to receiving the implant, twelve underwent stoma site ultrasonography at a median of 6 (range 1–10) months post operation, and none (0 of 12) had ultrasonographic evidence of a chronic seroma or fluid collection.

### 2.3.4 Serology

Serology results are illustrated in Figure 2.3. The white cell count was neither decreased nor elevated beyond the limits of the normal range in either group, with the exception of day 1 post-operation in the no-implant arm (Figure 2.3A). The erythrocyte sedimentation rate (Figure 2.3B) and C-reactive protein level (Figure 2.3C) were elevated beyond the upper limit of the normal range in both groups post-operatively, but there was no apparent difference between the groups.



**Figure 2.3 (A-C).** Serology results at varying time-points post stoma formation. (A), mean white cell count; (B), Mean erythrocyte sedimentation rate (ESR); (C), Mean C-reactive protein (CRP) level.

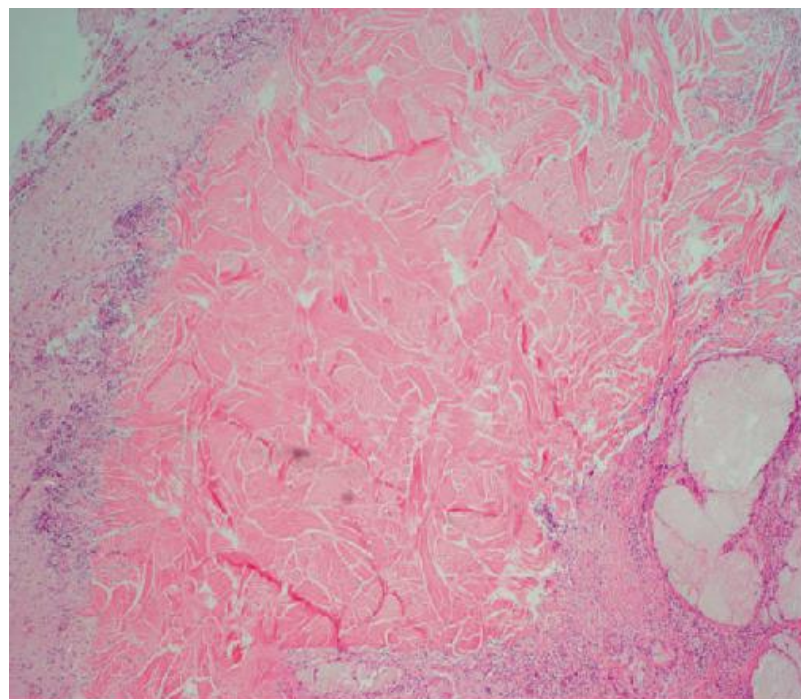
### 2.3.5 Microscopic findings

Eleven sets of biopsies were available for microscopic analysis.

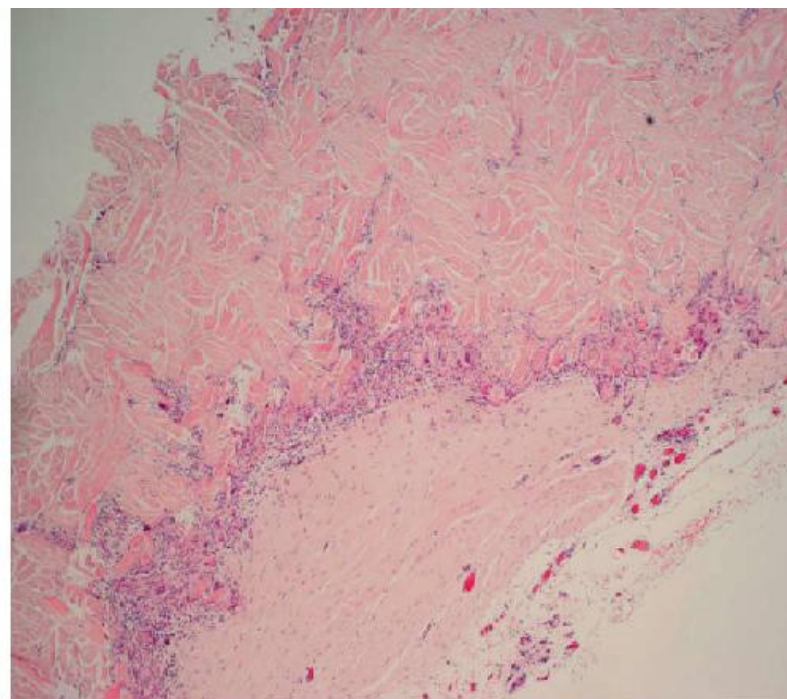
#### 2.3.5.1 Histology

Ten of the 11 sets of biopsies revealed a clear line of demarcation between the collagen implant and host connective tissue, with a mild mononuclear cell response and new vessel formation limited to the interface between the collagen implant and host connective tissue, and via native pores within the collagen implant (Figure 2.4). No polymorphonuclear cell response was evident, and the only foreign body giant cells were associated with stitch granulomas. There was focal evidence of organized and controlled host neo-collagen formation, albeit limited to regions of cellular infiltration and neovascularization (Figure 2.5). The collagen fibres paralleled the implant, with full-thickness penetration occurring via native pores. Host neo-collagen was clearly distinguishable from the distinctive collagen bundles associated with the implant.

The remaining biopsy, taken from a patient who had been treated with chemoradiation for a locally advanced rectal adenocarcinoma, showed a florid foreign body giant cell reaction resulting in localized destruction of the collagen implant.

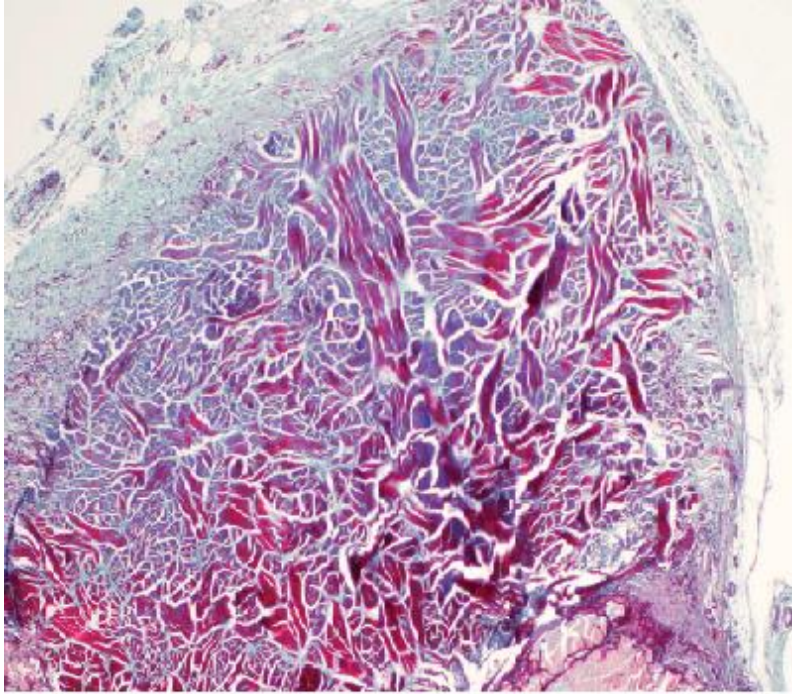


**A.** 1 month after implantation

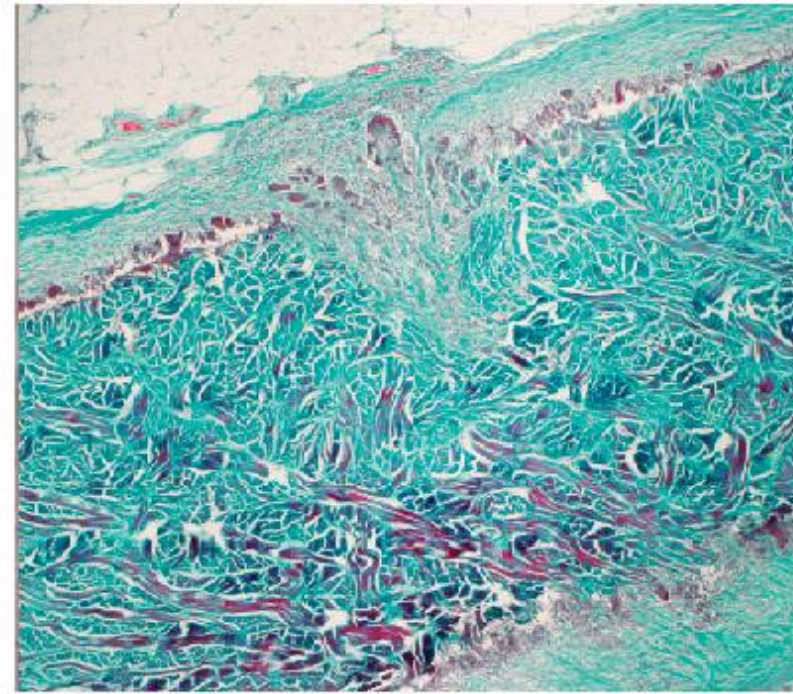


**B.** 6 months after implantation

**Figure 2.4** Haematoxylin and eosin stain of the implant and surrounding host tissue (A) at 1 month and (B) at 6 months. At 1 month a clear line of demarcation could be seen between the collagen implant and the host connective tissue, with a mild mononuclear cell response and new vessel formation limited to the interface between the implant and host connective tissue. At 6 months partial cellular infiltration was observed along the length of the implant (original magnification x40)



**A.** 1 month after implantation



**B.** 6 months after implantation

**Figure 2.5** Masson's trichromate stain of the implant and surrounding host tissue (A) at 1 month and (B) at 6 months. At 1 month organized host neo-collagen, distinct from the amorphous collagen bundles associated with the implant, could be seen running in parallel with the implant, with little integration. At 6 months there was focal evidence of host neo-collagen integrated with the implant (original magnification x40)

### 2.3.5.2 Immunohistochemistry

Results for markers of the inflammatory response are shown in Table 2.4. No B cells, natural killer cells, plasma cells or granulocytes were identified. There was a mild T cell response, with a similar proportion of T-helper cells to T-suppressor cells and a moderate macrophage response. All responses were limited to the interface between the implant and the host connective tissue, and native pores within the implant, with minimal apparent interindividual variability.

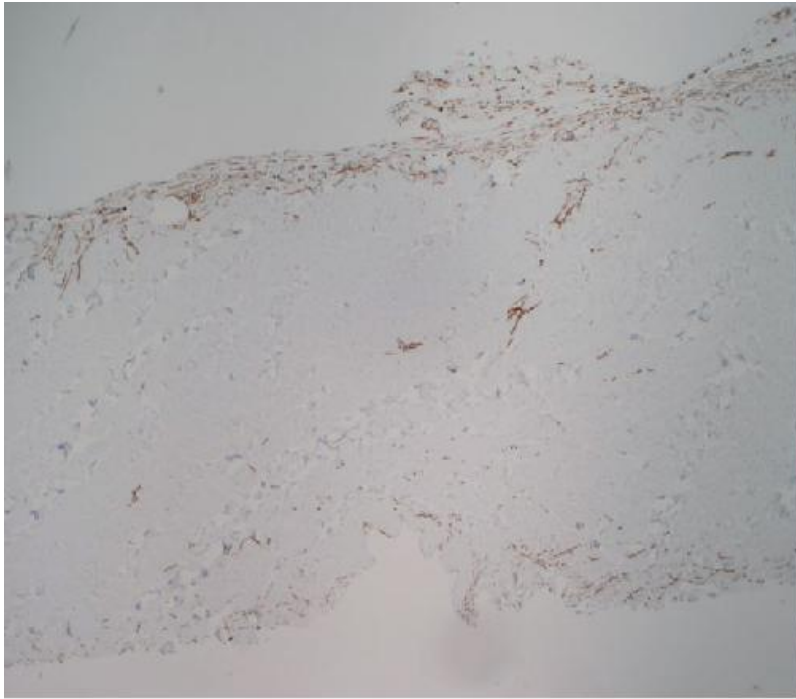
Over a median of 7 months *in vivo*, the expression of vimentin, a fibroblast marker, strongly increased within both the implant and the surrounding granulation tissue (Figure 2.6). Fibronectin and laminin expression (Figures 2.7 and 2.8 respectively), both adjacent to and within the implant, seemed proportional to the increasing expression of vimentin and CD68 (macrophage marker). Similarly, over time MMP-1 appeared to be expressed strongly around the mesenchymal cells within the Permacol<sup>®</sup> and surrounding tissue (Figure 2.9), although there was no evidence of implant degradation. All controls stained negatively.



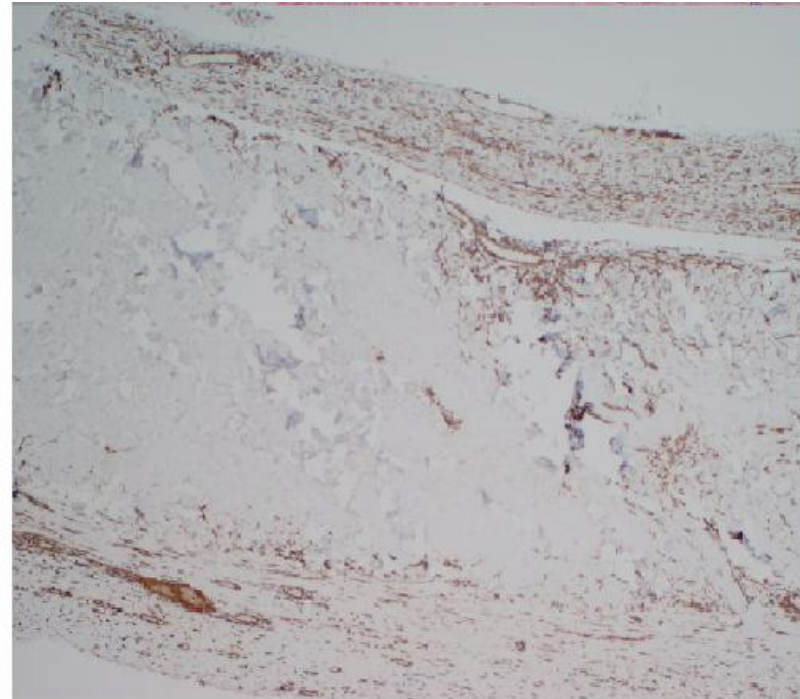
**Table 2.4** Inflammatory response to collagen implant

	<b>CD3</b>	<b>CD4</b>	<b>CD8</b>	<b>CD20</b>	<b>CD57</b>	<b>CD68</b>	<b>CD138</b>	<b>Myeloperoxidase</b>
<b>Within collagen implant</b>	0 (0 - 4)	0 (0 - 2)	0 (0 - 2)	0	0	0 (0 - 8)	0	0
<b>Native pores</b>	3 (0 - 12)	2 (0 - 8)	3 (0 - 15)	0 (0 - 12)	0	9 (0 - 22)	0 (0 - 4)	0 (0 - 1)
<b>Interface (between implant and host tissue)</b>	4 (0 - 32)	2 (0 - 9)	2 (0 - 17)	0 (0 - 3)	0	5.5 (0 - 36)	0 (0 - 4)	0 (0 - 7)

Values are median (range). The presence of various inflammatory cells was evaluated microscopically. Five fields per slide were counted at a magnification of x40 by two independent observers. Three slides per patient were analysed. These fields were selected randomly within the collagen implant itself, at the native pores within the implant, and at the interface between the implant and surrounding host tissue.

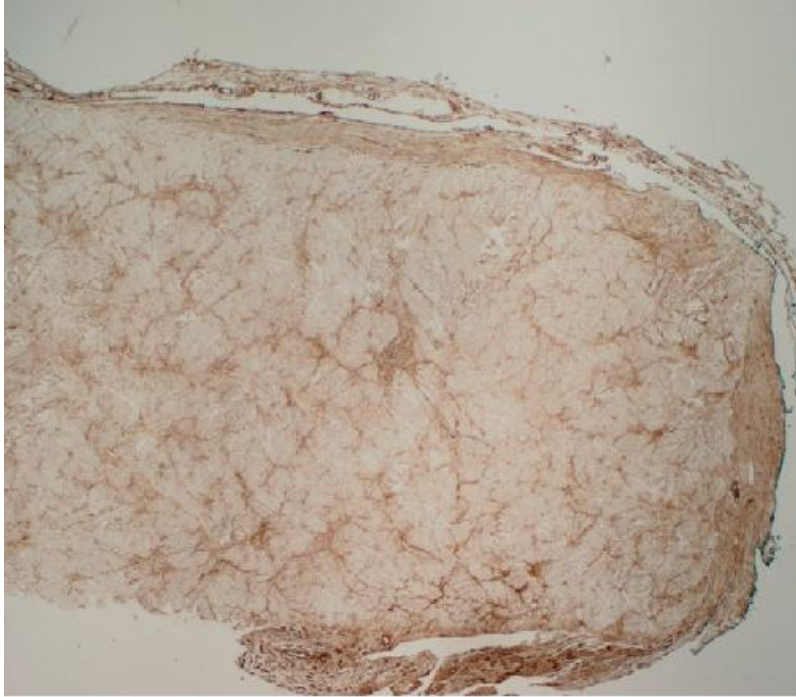


**A.** 1 month after implantation

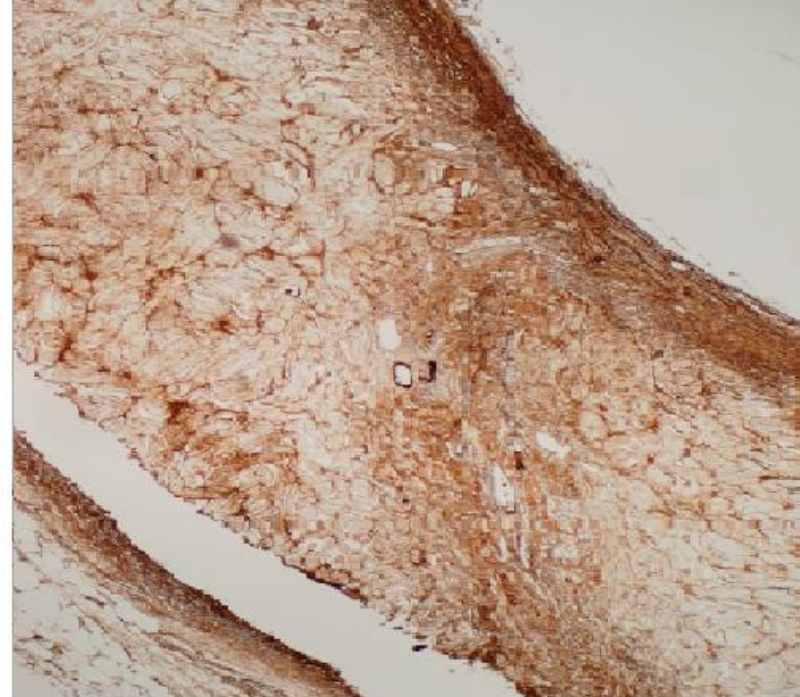


**B.** 6 months after implantation

**Figure 2.6** Vimentin stain of the implant and surrounding host tissue (A) at 1 month and (B) at 6 months. The strength and frequency of vimentin expression, a fibroblast marker, increased substantially from 1 to 6 months, both at the edges and infiltrating the implant (original magnification x40)



**A.** 1 month after implantation



**B.** 6 months after implantation

**Figure 2.7** Fibronectin stain of the implant and surrounding host tissue (A) at 1 month and (B) at 6 months. The strength and frequency of fibronectin expression increased substantially from 1 to 6 months, both at the edges and throughout the implant (original magnification x40)



**Figure 2.8** Laminin stain of the implant and surrounding host tissue at 6 months. Laminin both bordering and within the implant, and in conjunction with structures exhibiting typical vascular walls, was indicative of ongoing neovascularisation.



**Figure 2.9** Matrix metalloproteinase (MMP) 1 stain of the implant and surrounding host tissue at 6 months. MMP-1 expression at the edges and at focal points within the implant was proportional to the fibrovascular infiltration seen in previous figures.

## 2.4 Discussion

The view that prevention is the best approach to the management of parastomal hernias has been expressed in two review articles<sup>33;98</sup>, and there is good clinical evidence to date for placing a mesh at the time of stoma formation in order to achieve this aim<sup>122;123;125-127</sup>. However, the ideal material for this purpose and the most appropriate anatomical layer of the anterior abdominal wall within which to site the mesh has yet to be determined. Intuitively, the specific success determining characteristics of such a material would include: avoidance of a foreign body inflammatory response (biocompatibility), and therefore fibrosis, contraction and potential bowel erosion; adequate mechanical strength; and prolonged biodegradation, thereby avoiding herniation following early implant resorption. Furthermore, it has been proposed that both recurrent and incisional hernias (which by definition include parastomal hernias<sup>31</sup>) can be regarded as a consequence of a disturbed process in the wound healing pathway<sup>52;356</sup>. In the proliferation phase, type III collagen acts as a temporary scaffold for fibroblast attachment. Through a complex process of remodelling under the influence of the MMPs, this is replaced with type I collagen, which imparts long term strength. A shift of the ECM collagen ratio in favour of the 'immature' type III collagen may result in a loss of tensile strength, and predispose to hernia formation<sup>50</sup>. It is therefore reasonable to hypothesise that any biological material used to reinforce or repair the abdominal wall should either be predominantly composed of type I collagen or correct the balance of collagen metabolism.

Permacol<sup>®</sup> is composed of up to 95% type I collagen, and has been shown in both *in vitro* and animal studies to possess the aforementioned qualities. *In vitro* studies have shown that the cross-linking confers resistance to collagenase degradation<sup>180</sup>,

and when implanted into the abdominal wall of rat models, Permacol<sup>®</sup> induced a mild chronic inflammatory response with no evidence of significant fibrosis<sup>182;183</sup>. However, up until this study no evaluation of the human host response to this biomaterial had been performed.

The clinical results of this pilot trial suggest that this cross-linked collagen is safe to use: there were no complications related to infection or the proximity of the implant to the bowel, and there was no ultrasonographic evidence of localised chronic seroma formation or serological evidence of a systemic inflammatory response related specifically to the implant. Technically, the procedure is easy to perform and did not complicate stoma reversal. Most importantly, although there was no significant difference between the groups, there was a trend in favour of stoma reinforcement to prevent parastomal hernias, in that only one of fifteen patients who received the implant developed a parastomal hernia compared with three of ten patients who underwent a conventional stoma. The hernia that occurred in the implant arm of the trial, formed between the anterior layer of the rectus sheath and the implant itself, which highlights one of the main concerns of the fascial onlay technique, and led to the subsequent placement of the implant in the pre-peritoneal space. No hernias developed in this sub group of patients. It could be argued that all patients should have undergone post-operative CT imaging, the gold standard for parastomal hernia imaging. However, CT is only indicated in those patients whose symptoms are suggestive of a hernia, but in whom this cannot be clinically demonstrated<sup>43</sup>. In this regard, the two patients in the implant arm of the trial with symptoms suggestive of a hernia (one of whom complained of a parastomal bulge and the other nausea, vomiting and abdominal bloating) did not undergo CT imaging as a decision was made to determine whether a hernia was present at the time of stoma reversal. Additionally, it is acknowledged that Permacol<sup>®</sup> costs significantly more than Vypro<sup>®</sup>

(£770 per 10x10cm, 1.0mm thick, sheet compared to ~ £60 per 10x15cm sheet), the mesh employed by Janes *et al* in the only published randomised controlled trial for parastomal hernia prevention to date<sup>126</sup>. Further appropriately powered studies will have to be undertaken to assess not only the efficacy of Permacol<sup>®</sup> reinforcement of stomas compared to conventional stomas, but also the efficacy, safety and economic cost-benefit of Permacol<sup>®</sup> over large pore light-weight polypropylene meshes in the prevention of parastomal hernias.

The decision to pilot the technique on defunctioning loop stomas was based on a number of factors. Previous studies have demonstrated a 6% herniation rate in loop stomas at 3 months<sup>33</sup>, and it is reasonable to assume that the rate increases with the duration of follow-up, as has been shown with end stomas<sup>98</sup>. Moreover, the construction of loop stomas requires a comparatively larger abdominal trephine than end stomas, which theoretically places them at greater risk of developing a parastomal hernia in the longer term. These are important points when considering that the median time to stoma reversal in those patients who underwent stoma reversal was 6.5 (range 1–10) months, and that the remainder, either being unsuitable or unwilling to undergo reversal, are therefore at increased risk of herniation in the longer term. Other factors included the greater technical ease of reversing loop stomas, compared to end stomas, in the event of complications, and the unique opportunity this study model provided for histological assessment of the human host response to the implant.

The histological data demonstrate that, in this setting of parastomal hernia prevention, Permacol<sup>®</sup> has excellent biocompatibility and resistance to degradation. At a median of 7 months *in vivo* Permacol<sup>®</sup> induced a mild-to-moderate non-foreign body inflammatory response with no evidence of fibrosis or implant contraction, and underwent minimal implant degradation. There was evidence of increasing fibroblast

integration, proliferation, synthesis of neo-ECM proteins (fibronectin, laminin and MMP-1) and neovascularization at the periphery of the implant and via native pores. Neo-collagen deposition occurred in an organized pattern, the collagen fibres paralleling the implant both at the visceral and parietal surfaces, presumably resulting in greater mechanical tissue strength than when originally implanted. Although no tensiometer studies were performed, a previous study of this material confirmed that it provides adequate and durable prosthetic–native tissue tensile strength for use as an hernia prosthesis<sup>183</sup>. The present observations also concur with findings *in vitro* and in animal model studies<sup>180-182;184-186</sup>, and further support the application of the implant for soft tissue reinforcement, especially when retention of mechanical strength is desirable and bowel proximity is a concern. In this context, the results suggest that the implant not only has the potential for safe and effective use in the extraperitoneal management of parastomal hernias, but also supports the role of laparoscopic intraperitoneal placement of this mesh for parastomal and other incisional hernias.

Although the data indicate that the implant exhibits a number of the crucial requirements of an abdominal wall repair material, only limited (rather than full thickness) fibrovascular ingrowth was observed. For implants to fulfil a repair function a balance must be struck between implant degradation, cellular infiltration and neovascularization, and subsequent formation of a neo-ECM. The implant's biocompatibility and prolonged biodegradation is largely dependent on isocyanate-induced cross-links between the polypeptide chains. These have the dual function of suppressing biodegradation, by inhibiting polymorphonuclear cell phagocytosis and resisting MMP activity, and consequently improving biocompatibility by reducing the availability of cleaved antigenic molecular components. It is this resistance to the action of MMPs that mostly probably limits fibrovascular ingrowth<sup>357</sup>, although the



increasing deposition of MMP-1 observed over the study period suggests that further fibrovascular integration may occur with longer follow-up. Two rodent model studies comparing Permacol<sup>®</sup> with a non-cross-linked porcine derived alternative (Surgisis<sup>®</sup>), showed that although Surgisis<sup>®</sup> demonstrated a significantly greater degree of fibrovascular integration and collagen deposition at 9-weeks<sup>187</sup>, there was no difference at 20-weeks<sup>185</sup>.

Increasing the porosity of a biomaterial has been shown to increase subsequent cellular ingrowth and neovascularisation, the pore size of the biomaterial being critical to its performance<sup>358</sup>. A further consideration is the distance of cells more than 200µm from a blood vessel being prone to hypoxia and limitation of other nutrients<sup>358</sup>. However, attempts to increase the rate and degree of vascularisation of Permacol<sup>®</sup>, by increasing porosity with a diamond CO<sub>2</sub> laser and topical application of vascular endothelial growth factor (VEGF), demonstrated that vascular ingrowth was still limited to the laser pores alone and pre-soaking the implant in VEGF did not influence the vascularity of the surrounding material<sup>40</sup>. Studies employing alternate cross-linked biomaterials have shown that the time taken for full-thickness fibrovascular integration increases proportionally with the percentage cross-linkage of the implant<sup>359</sup>, and therefore although Permacol<sup>®</sup> has excellent potential for soft tissue reinforcement, modifications (such as reducing the degree of cross-linkage) may be required if more rapid full-thickness cellular integration is deemed necessary for its intended purpose.

In conclusion, using a cross-linked xenogeneic collagen implant to prevent parastomal hernias seems safe (especially in regards to bowel related complications), technically feasible and is potentially efficacious. Further study, employing appropriately powered sample sizes, longer-term follow-up and cost-benefit analysis, is now required to establish whether this implant is at least as

effective as synthetic mesh at preventing parastomal herniation, and which is associated with the fewest complications and provides the best economic cost-benefit. In this respect, and as a progression of the pilot study, a multi-centre randomized controlled trial (Permacol<sup>®</sup> Reinforcement of Permanent Stomas Versus Standard Technique in Reduction of Parastomal Hernia, PROPHECI) aiming to prospectively recruit 300 patients is currently in progress. An additional challenge will be to identify whether all patients undergoing stoma formation should undergo prophylactic primary mesh placement or if the procedure should be targeted at those most at risk of such a complication.

## **2.5 Permission to reprint**

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# Chapter 3

The Snug Seton: short and medium term results of slow  
fistulotomy for idiopathic anal fistulas

### 3.1 Introduction

The treatment of anal fistulas is necessarily diverse, not least because no single technique is universally effective. To date, fistulotomy remains the most effective way of eradicating the pathology, but division of those sphincter muscle fibres enclosed by the tract, renders the patient at risk of faecal incontinence, with reported rates ranging from 5 to 40%<sup>255</sup>. A prospective study of the effects of fistulotomy has revealed that even division of the internal anal sphincter alone is associated with a significant incidence of functional impairment<sup>250</sup>. Fistulotomy is thus usually reserved for those patients in whom the consequences of sphincter division are anticipated to result in minimal functional disturbance, i.e. 'low' fistulas, as interpreted by the individual surgeon, and those patients with good pre-operative function and strong anal sphincters. For those in whom fistulotomy is not recommended (including those in whom even minor degrees of incontinence would be unacceptable), alternative strategies exist including 'sphincter conserving' methods or the placement of a long-term loose draining seton. The former have outcomes that are relatively poor in terms of fistula persistence<sup>275;297;302;307</sup> and the latter, whilst not placing the sphincter at risk, is simply palliative<sup>248</sup>. A study of the loose seton technique for healing transsphincteric fistulae (in which the external anal sphincter is preserved) has revealed significant recurrence rates over the long-term<sup>268</sup>.

An alternative to one-stage fistulotomy is the tight or cutting seton, by which the muscle enclosed by the seton is more slowly divided. Although several studies have reported successful eradication of 'high' fistulas using this method, the majority have unacceptable rates of both frequency and severity of anal incontinence<sup>270;272-275;277-290</sup>, especially when the interval between tightening is short<sup>288</sup>. Thus, the cutting

seton is not recommended in 'high' fistulas or patients predisposed to continence disturbance<sup>274</sup>.

A modification of the cutting seton technique was therefore conceived, based upon the following principles:

- Reduced continence disturbance, effected by a slower gradual severance of tissue (it seeming intuitive that the incidence and severity of incontinence might relate to the speed with which sphincter division is effected: the slower the division, the lower the risk to continence<sup>222;274</sup>).
- The lack of requirement for replacement or re-tightening, and minimal patient discomfort, thereby allowing early return to normal activity, due to the elastic nature of the seton which ensures it slowly migrates caudally.

The aim of this study was to assess the short and intermediate outcomes of this technique.

## **3.2 Patients and methods**

### **3.2.1 Patients**

Patients were selected from those undergoing surgery for anal fistula by a single Consultant Surgeon at 2 hospitals over a 5-year period, during which a total of 191 anal fistula operations were performed. These included one-stage fistulotomy, palliation by long-term loose seton and the snug seton technique.

The snug seton technique was offered to patients based on the following criteria:

- The fistula aetiology was deemed cryptoglandular.
- The patient was keen for fistula eradication, rather than palliation.
- No symptoms of incontinence, but a substantial threat to continence posed by conventional fistulotomy.

- Acute sepsis and secondary tracts, if present, had been dealt with adequately previously, leaving a single primary tract (as with all 'advanced' techniques).

### 3.2.2 Snug seton surgical technique

Informed consent was obtained from all patients. They were given appropriate thromboembolic prophylaxis and a pre-operative phosphate enema. Detailed examination was performed under anaesthesia (EUA) in the lithotomy position and the fistula characterized according to both Parks' classification<sup>215;228</sup>, and for transsphincteric fistulas, the level defined according to where the tract crossed the external anal sphincter (EAS) in relation to the dentate line (high, mid (at the level of the dentate line) or low). The extrasphincteric component of the primary tract was either excised by core fistulectomy, or laid open, using diathermy. The primary tract traversing the sphincter was thoroughly curetted if granulation tissue was present, and cored out if epithelialized. Excised tracts were sent for routine histopathological analysis. Sharp division of the skin and anoderm was performed to denude the sphincter below the tract, but with no internal sphincter division. A 1 mm silastic seton (silicone nerve vessel retractor, Medasil<sup>®</sup>) was drawn into position using a 0/0 nylon suture, passed along a grooved fistula probe. This was then 'snugly' tied around the sphincter muscle, so that it abutted the enclosed tissue, but with only minimal tension. A Spongostan<sup>®</sup> (Johnson & Johnson Medical Ltd, Skipton, UK) intra-anal dressing was inserted, and Kaltostat<sup>®</sup> lightly tucked into any external wound. Gauze, dressing pad and mesh pants were used to support the dressing.

Post-operatively, patients received regular Milpar, Fybogel, Paracetamol and Diclofenac (if not contraindicated). Patients were discharged home when comfortable, usually the evening of surgery or the following morning, depending on the size of the external wound. When necessary, patients had a daily change of

wound dressing performed by a district nurse. In those in whom it was evident that the seton would not completely cut through spontaneously, patients were subsequently admitted as a day case, for EUA and division of the remnant of tissue enclosed by the seton (on occasions involving a few subcutaneous EAS fibres). Follow-up was performed on a regular basis until the seton had come out (spontaneously or surgically released), the fistula was deemed to have been eradicated and all wounds had satisfactorily healed.

### 3.2.3 Data collection

The study was approved by the local ethics committee. Short-term assessment was performed by case note review to ascertain:

- The proportion of patients in which the seton cut out completely, without intervention, and the time to achieve this;
- The proportion of patients requiring division of residual enclosed tissue;
- The proportion of patients with fistula healing, determined by documented symptom resolution and no clinical evidence of fistula persistence;
- The proportion of patients with initial continence disturbance (and severity thereof).

Medium-term assessment was performed by mailing to each patient an invitation to participate with an attached questionnaire, as described by Garcia-Aguilar *et al* (see appendix)<sup>360</sup>. Patients were contacted two weeks later by telephone, in order to record the results. This was performed at a median duration of 42 months (range 10–64 months) after the seton had either cut through or the residual enclosed tissue laid open. The questionnaire specifically assessed: the initial success of the procedure, time taken to return to work and for the perianal wound to heal, symptoms associated with recurrence (perianal pain, swelling or discharge),

continence disturbance (type, grade and duration), the necessity of wearing a pad, patient satisfaction and lifestyle alteration caused by the incontinence. Anal incontinence was defined as any reported difficulty holding gas, soiling of underwear, or accidental bowel movements since surgery<sup>360</sup>.

### **3.2.4 Statistical analysis**

Comparisons were made between the time taken for the seton to cut through intersphincteric vs transsphincteric fistulas (Mann–Whitney U-test), and the levels (high, mid and low) of transsphincteric fistulas (Kruskal–Wallis one-way ANOVA). Analysis was performed using a commercially available software package (Prism 4, Graffpad software, San Diego, CA, USA). Statistical significance was assigned at the 5% level.

## **3.3 Results**

During the study period, 35 patients underwent the snug seton technique.

### **3.3.1 Short-term assessment**

Six patients were not included in the analysis. One patient died from an unrelated cause before the seton had cut out; one patient's seton fell out prematurely through knot slippage, and declined re-insertion; and the remaining four patients' case notes could not be retrieved. Therefore, 29 patients' notes (median age: 42 years, range 26–70 years; 3 female) were available for short term analysis. Seven patients had undergone previous fistula surgery (5 fistulotomies, 2 core-out and loose seton placements), one had previously undergone manual anal dilatation, and two women had experienced obstetric trauma requiring suture repair. However none had any symptoms of anal incontinence prior to treatment. Fistulas were classified intra-



operatively as 9 intersphincteric and 20 transsphincteric (5 high, 6 mid, 9 low). Histological analysis revealed no features suggestive of a specific aetiology in any patient.

The results are summarised in Table 3.1. All fistulas (100%) were reported as healed. There were no episodes of major incontinence (frank faecal incontinence). Ten (34%) patients (1 female; 5 previous surgery; 8 transsphincteric; 3 low, 3 mid, 2 high; 2 intersphincteric) experienced minor continence disturbance (occasional soiling of underwear and/or flatus incontinence). Other complications included one patient complaining of occasional pain on defaecation secondary to development of a superficial fissure in the anal scar, one complaining of pruritus in the region of the scar tissue, and one patient who developed an abscess lateral to the external opening of the fistula 12 weeks post snug seton insertion. This was treated by incision and drainage with reinsertion of snug seton, after which no further complications developed.

The time taken for the seton to cut through intersphincteric fistulas (median 7, range 2–24, weeks) was significantly shorter than for transsphincteric fistulas (median 26, range 1–164, weeks,  $P = 0.004$ ) (Fig. 3.1A). Similarly, the time taken for the seton to cut through transsphincteric fistulas was significantly related to the level of the fistula: low, median 10 (range 1–38) weeks; mid, median 31 (range 25–80) weeks; high, median 84 (range 20–164) weeks ( $P = 0.045$ ) (Fig. 3.1B).

### **3.3.2 Medium-term assessment**

Of 29 patients in whom short-term data were available, one patient declined to participate, and 12 could not be contacted. Therefore 16 patients (3 female) were reassessed: 4 intersphincteric, 12 transsphincteric fistulae (2 high, 4 mid, 6 low). The

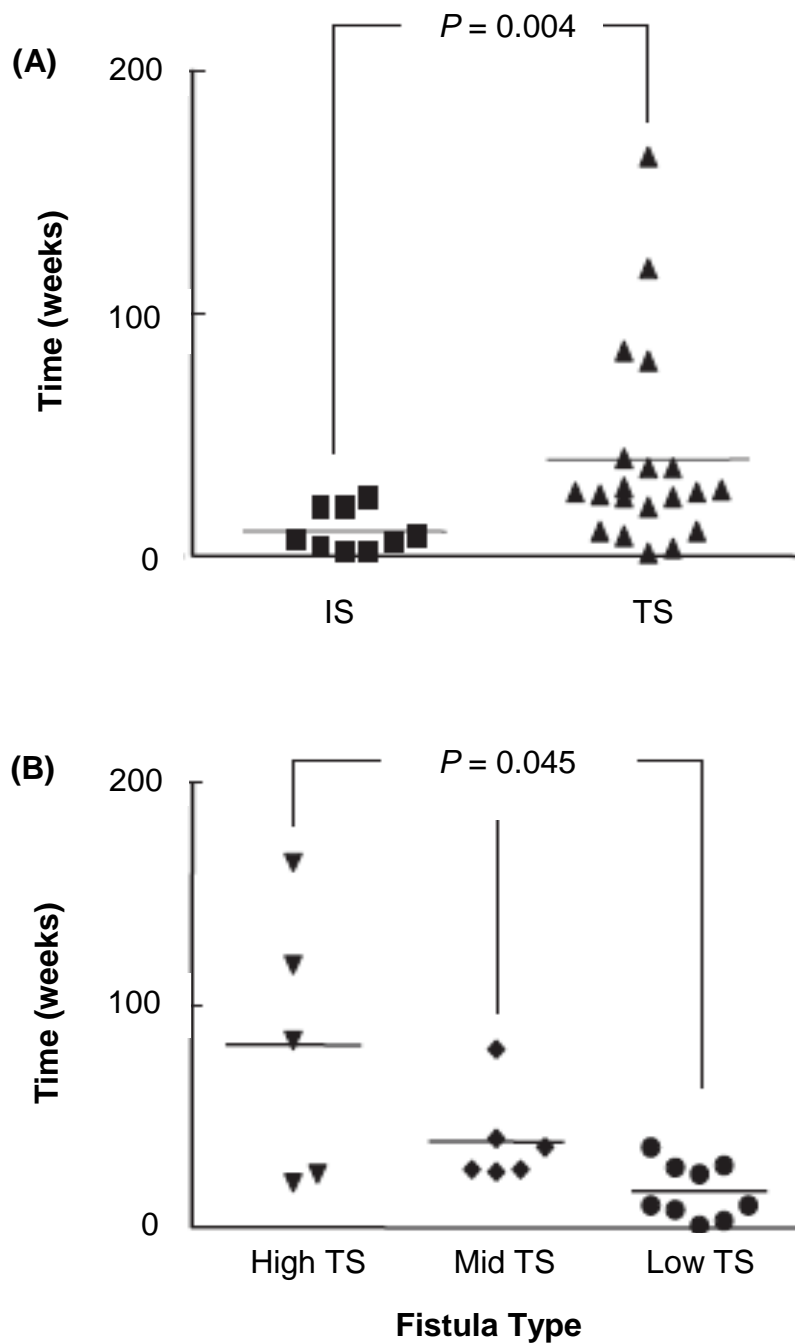
results are shown in Table 3.2. No patient suffered recurrence, but minor incontinence persisted in 4 / 16 (25%) patients (0 females; 2 previous surgery; 3 transsphincteric - 2 low, 1 mid; 1 intersphincteric). Two patients (12.5%) felt that their lifestyle had been adversely affected (both of whom had permanent minor continence disturbance), however, all patients were either 'satisfied' or 'very satisfied' with the procedure and its outcome (Table 3.3).

**Table 3.1** Short-term assessment (n = 29)

	<b>No. of patients (%) or median value (range)</b>
<b>No. of patients in whom seton cut-out without further intervention</b>	15 (52%)
<b>Time to cut-out without further intervention in weeks</b>	Median 24 (1 – 164)
<b>No. of patients requiring division of residual tissue</b>	14 (48%)
<b>Time to further intervention in weeks</b>	Median 35 (6–118)
<b>No. of patients whose fistula healed</b>	29 (100%)
<b>Initial continence disturbance</b>	10 (34%)
<b>In <i>transsphincteric fistulas</i></b>	8/20 (40%)
<b>In <i>intersphincteric fistulas</i></b>	2/9 (22%)

**Table 3.2** Medium-term assessment (n = 16)

	<b>No. of patients (%) or median value (range)</b>
<b>Time to perianal wound healing in weeks</b>	Median 3 (1 – 24)
<b>Time to return to work in weeks</b>	Median 2 (0 – 8)
<b>No. of patients satisfied</b>	
<i>Very satisfied</i>	11/ 16 (69%)
<i>Satisfied</i>	5/ 16 (31%)
<b>No. of patients with fistula recurrence</b>	0 (0%)
<b>No. of patients with initial continence disturbance:</b>	
<i>In transsphincteric fistulas</i>	7/ 16 (44%)
<i>In intersphincteric fistulas</i>	5/ 12 (42%)
<i>In intersphincteric fistulas</i>	2/ 4 (50%)
<b>No. of patients with persistent continence disturbance:</b>	
<i>In transsphincteric fistulas</i>	4/ 16 (25%)
<i>In intersphincteric fistulas</i>	3/ 12 (25%)
<i>In intersphincteric fistulas</i>	1/ 4 (25%)



**Figure 3.1** (A) Time taken for seton to cut through the enclosed tissue within an intersphincteric (IS) vs transsphincteric (TS) fistula.  $P = 0.004$  (Mann–Whitney U-test). The horizontal lines represent the median values for each fistula type. (B) Time taken for seton to cut through the enclosed tissue vs level of transsphincteric (TS) fistula.  $P = 0.0445$  (Kruskal–Wallis one-way ANOVA). The horizontal lines represent the median values for each fistula level.

**Table 3.3** Effect on lifestyle at medium-term assessment (n = 16)

	<b>Physical activities</b>	<b>Social activities</b>	<b>Sexual activities</b>
<b>Not at all</b>	16 (100%)	15 (94%)	15 (94%)
<b>To some extent</b>	0	0	1 (6%)
<b>Greatly</b>	0	1 (6%)	0

### 3.4 Discussion

Anal fistula management can be problematic; both the individual fistula anatomy and the amount of sphincter across which it passes are highly variable, as is the individual sufferer's expectations of treatment (often dependent upon the duration and severity of symptoms, and the number and types of previous attempts at eradication). The number of different approaches attests to the failure of any one technique to achieve the dual aims of permanent fistula eradication and the preservation of sphincteric function. For most people, eradication of symptoms is the primary goal, and fistulotomy is the gold standard in this respect. Nevertheless, there is a functional price to pay, even for 'simple, low' fistulas such as distal intersphincteric<sup>250</sup>, just as there is for lateral internal sphincterotomy, the questionable gold standard surgical treatment for chronic anal fissure<sup>361</sup>.

The traditional use of the loose seton in eradicating transsphincteric fistulas has two drawbacks. Firstly, the internal sphincter is divided so as to eradicate the presumed infecting source (the diseased anal gland in the intersphincteric space); and secondly, initial cure rates of around 50% are compromised by a cumulative

recurrence rate over time<sup>268</sup>. Cutting or tight setons in contrast, replicate the role of fistulotomy in terms of fistula eradication, but also carry the functional risks of sphincter division<sup>270;272-275;277-286;288-290</sup>. The rates of incontinence associated with the conventional cutting seton are likely to be proportional to the speed of sphincter division. Additionally there is the need for repeated replacement/ tightening of the cutting material, unless ingenious appendages are added such that thigh flexion and extension effect the cutting<sup>362</sup>.

The chemical seton, in which the enclosed tissue is divided by the caustic nature of the thread (at a rate of approximately 1 cm every 6 days), when compared to fistulotomy has been shown to have no difference in rates of incontinence or recurrence, but because the trial designs excluded all those with anything but low transsphincteric fistulas, we do not know whether such a technique has a role for the more problematic higher fistula<sup>293;294</sup>.

The anal sphincter mechanism consists not simply of the internal and external sphincters, but also of a complex supporting meshwork derived from the conjoint longitudinal muscle layer, which holds these muscles in place, and also gives attachment to skin, anal canal mucosa and fascia laterally at the pelvic side walls<sup>222</sup>. Division of the anal sphincter complex thus involves division of this supporting framework. The main theory behind the development of the 'snug' seton was that disruption to this framework, and therefore of separation of those muscles it supported would be minimized. Furthermore, the use of an elastic material such as silastic would avoid the need for repeated replacement or tightening, and be relatively comfortable, especially if the underlying sensitive skin and anoderm had been divided.

The results of this procedure, for the treatment of 35 patients with idiopathic anal fistula, have been presented. Clearly, the design of the study (retrospective review)

has limitations, and mailed and telephone questionnaires are subject to criticism. It has been shown that fewer mild symptoms are reported by telephone than by mail questionnaire<sup>363</sup>, and missing data are more frequent for mailed questionnaires than for telephone interviews<sup>364</sup>. The response rate of 55%, at medium-term review, is disappointing, and it is therefore possible that data from the remainder of patients may have significantly impacted on the medium-term results. However, the response rate does reflect the relatively young and highly migrant population within Tower Hamlets and Hackney<sup>365</sup>. Additionally, the preponderance of male patients (26 of 29) is a function of the natural preponderance of anal fistulas in males<sup>210</sup>, and the exclusion of those female patients who may have suffered a disturbance to continence secondary to obstetric trauma.

Nevertheless, based on the data presented, the technique has a recurrence rate of 0%, and short and medium term rates of minor continence disturbance of 34% and 25%, respectively, with all patients being at least satisfied with their outcome at medium-term review. These results merit comparison with those available from other procedures. However, difficulties clearly exist with such comparisons in relation to fistula aetiology, level or complexity, age, gender, previous anal trauma, as well as the methods of assessment. Thus the question of whether, for example, a one-stage fistulotomy or traditional cutting seton are superior to the snug seton remains. An appropriately constructed prospective randomised controlled trial, employing endoanal ultrasound, or preferably MRI using an endoanal coil, to quantify and compare the degrees of internal and external sphincter disruption and separation incurred, might give the answer<sup>366;367</sup>. However, the risks to continence of these techniques, in the majority of this cohort, would make such a trial debatably unethical, and the results of these approaches from the relevant literature support this stance<sup>255;275</sup>. Similarly, the results of this study in terms of fistula eradication

would also make it difficult to justify, by strict scientific methodology, comparison with sphincter conserving techniques such as mucosal advancement flaps or fibrin glue, in which efforts to retain function are compromised by poorer cure rates<sup>302,307</sup>. The use of a silastic cutting seton in the management of transsphincteric fistulas has been reported previously, but in that study of 17 patients, initial internal sphincterotomy was performed, subsequent tightening was achieved by the application of Barron's haemorrhoidal bands, and the authors admitted that external sphincter involvement (< 40%) was less than that in patients treated by loose setons or advancement flaps<sup>275</sup>. A technique involving an alternative 'elastic' seton, fashioned from the wrist of a surgical glove, has been described which similarly relies on the natural recoil of the seton to avoid any need for further tightenings<sup>276</sup>. However the mean time taken for the seton to cut through was 2.5 (range 1.5-4) weeks, which implies a tight rather than snug application, and a 20% reduction in baseline continence scores in treated patients further emphasises the need for a slow severance of enclosed sphincteric muscle.

There was clearly a wide spectrum of time to sphincter division in this study. It could be argued that there is no quantitative assessment of the degree of tension imposed by the silastic seton. The aim was that muscle division takes place as slowly as possible by snugly applying the seton, so that it abuts the enclosed sphincteric tissue, with only minimal tension. The relations between fistula classification and level, and time to cut through, indicate that similar tension was in fact applied amongst the patients. Numbers were too small to determine relations of sex, previous anal surgery/trauma, fistula classification or level, time for the seton to cut through or lay open of residual enclosed tissue, and postoperative continence. Knot failure in one patient has led to subsequent knot securement with a reinforcing 0/0 Ethibond™ (Ethicon™, Johnson & Johnson International) thread.



It might be argued that patients should have undergone both pre-operative magnetic resonance imaging (MRI), the gold standard for anal fistula imaging<sup>233;368-370</sup>, and anorectal physiological testing. However, MRI is not clinically indicated in the majority of cases of anal fistula, in whom fistula topography may be accurately obtained clinically, or at surgery, by an experienced fistula surgeon (in this case, the senior trial investigator and supervisor of this thesis). The fact that the snug seton method was successful in fistula eradication, in all cases when assessed at medium-term review, attests to this. Nevertheless, the importance of converting a fistula, complicated by secondary extensions or collections, to a single primary tract (with the external opening adjacent to the denuded sphincteric component), cannot be overstated, and if there is uncertainty then MRI, whose clinical usefulness lies mainly in the detection of secondary extensions, should be employed. The results of pre-operative physiological assessment may identify patients at risk<sup>249</sup>, but have been shown in a prospective study, not in fact, to predict postoperative physiological or functional outcomes<sup>250</sup>. In that study, disturbances in postoperative continence appeared to relate mainly to anodermal sensitivity and reduced postoperative resting pressure profiles (rather than squeeze pressures). The latter relates predominantly to internal anal sphincter division (presumably in terms of both length and width of the resultant defect), and the former to scarring, relating more to the proportion of the anal luminal circumference affected, especially after lay open of transsphincteric fistulas. In such situations, the degree of separation of the divided mucosa/anoderm would presumably be greater, incurred by division of both main sphincteric muscle components.

Although in the short term, the majority of patients have previously been shown to be happy to put up with 'minor' degrees of incontinence as a reasonable price to pay to be rid of sepsis<sup>250</sup>, and that with time continence improves in some patients, a

proportion will remain in status quo or may in fact in the longer term deteriorate<sup>371</sup>. The technique of the snug seton goes some way to improving the functional outcome for such patients, without compromising on the rates of fistula eradication achieved by more established 'lay open' techniques, and merits addition to the fistula surgeon's list of possible surgical approaches. However these purely surgical strategies, irrespective of novel adaptations to technique or material, by their very nature will always require fistula eradication and maintenance of continence to be directly competing priorities. The recent use of strategies employing modern biomaterials aimed at tissue repair, rather than minimizing destruction, although not yet as efficacious as their traditional counterparts, represent a great leap forward and offer the most likely path to achieving the fistula surgeons' panacea.

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# Chapter 4

Management of Idiopathic Anal Fistula Using Cross-linked Collagen: A prospective phase 1 study

## 4.1 Introduction

The balance between fistula eradication and preservation of continence has already been addressed. Biomaterials in the form of fibrin glue and an anal fistula plug, composed of lyophilised porcine small intestine submucosa (Surgisis<sup>®</sup>, Cook Surgical, Bloomington, USA), have been recently used as part of novel strategies to promote fistula healing whilst avoiding sphincter disruption<sup>307;333</sup>. Both have similar widely variable initial success rates, which decrease with the duration of follow-up<sup>263;306-310;334;337-345</sup>. Proposed reasons for their failure include those associated with recurrence after conventional therapy, early extrusion of the biomaterial, and those specific to their biology<sup>318;336</sup>. Deemed critical to the success of all traditional sphincter sparing techniques are the elimination of acute sepsis, the eradication of secondary extensions, and the adequate removal of all granulation or epithelial tissue lining the tract<sup>227;251;253</sup>. Interestingly, only the former is recommended by a published consensus statement on the optimal management protocol for the AFP<sup>335</sup>. Biologically, fibrin glue's limitations are two-fold: it seems to encourage epithelialisation across the openings of the fistula, rather than actual fistula healing, with the former often misinterpreted as evidence of the latter<sup>252</sup>; and fibrin glue is resorbed within five to ten days, which is insufficient time to act as a scaffold for tissue repair<sup>325</sup>. In contrast, porcine small intestine submucosa has been shown to successfully support tissue repair in clean environments, however there are conflicting reports as to whether it can reproduce such success in contaminated fields, due to relatively high rates of bacterial colonisation and subsequent premature lysis<sup>77;166;169;170;372</sup>.

An alternative biomaterial is Permacol<sup>®</sup> (Tissue Science Laboratories Plc, Aldershot, UK), a porcine-derived acellular dermal collagen, which is cross-linked to prevent early enzymatic degradation<sup>180</sup>. It is available as a solid implant and as a milled fibre

suspension, and both preparations would seem to offer advantages for fistula repair. The solid implant can be easily sited and fashioned to the dimensions of a fistula, in a similar manner to the anal fistula plug, but as demonstrated in chapter 2, although biocompatible, full thickness host tissue integration can be prolonged. The milled fibres need to be retained within the fistula, and for this purpose could be suspended in fibrin glue. Following epithelialisation of the tract openings and rapid glue resorption, the remaining collagen fibres provide a robust network of bioscaffolds with the potential for rapid host tissue integration.

The aims of this phase 1 trial were to assess the safety, feasibility and potential efficacy of using this cross-linked collagen, as either a solid implant or as milled fibres suspended in fibrin glue, to treat idiopathic anal fistulas.

## **4.2 Methods and materials**

The study was approved by the local ethics committee (REC reference: P/03/870), and informed consent was obtained from all patients participating in the study.

### **4.2.1 Selection criteria**

Consecutive patients with an anal fistula deemed idiopathic, under the care of a single surgeon, and in whom fistulotomy was deemed unsuitable (on the basis of the fistula type and level, threat to continence or patient choice) were prospectively invited to participate in the study. Invited patients were provided with an information sheet detailing pre-operative assessment, the aims and potential complications of treatment, and follow up arrangements.

#### **4.2.2 Pre-operative assessment**

Patients underwent pre-operative symptom and continence assessment, and clinical examination. Patients also underwent station pull-through anal manometry to determine resting and squeeze pressures along the length of the anal sphincter, endoanal ultrasound (EAUS) to assess sphincter integrity, and magnetic resonance imaging (MRI) using standard anal fistula sequencing to determine fistula anatomy<sup>237</sup>. Patients with either clinical or radiological evidence of secondary tracts or acute sepsis were excluded from the trial until these had been eradicated, leaving a single (usually loose seton drained) primary tract.

#### **4.2.3 Materials**

Approval for the use of all materials had been obtained from the Medicines Control Agency.

##### **4.2.3.1 Permacol<sup>®</sup>**

Biological and manufacturing details as per Chapter 2. The solid implant was fashioned from sterile sheets 1.0 mm in thickness. The Permacol injection<sup>®</sup> (Tissue Science Laboratories Plc), is a 2.5 ml 60% (wet weight/volume) suspension in saline of the cryogenically milled implant, with a defined particle size of 150 µm in diameter. Tissue Science Laboratories Plc unconditionally donated both materials.

##### **4.2.3.2 Fibrin glue**

The 1.0 ml Tisseel Kit<sup>®</sup> -Two Component Fibrin Sealant (Baxter Healthcare Ltd, Newbury UK) was employed.

#### **4.2.4 Surgical technique**

Patients were given venous thromboembolic prophylaxis, but no specific bowel preparation or perioperative antibiotics. Examination under anaesthesia was performed in the lithotomy position. Following confirmation of a single primary tract and position of the internal opening using a variety of angled fistula probes, the fistula was characterised according to Parks' classification<sup>228</sup>. For trans-sphincteric fistulas the level was defined according to where the tract crossed the external anal sphincter in relation to the dentate line (high, mid [at the level of the dentate line] or low)<sup>373</sup>. The extrasphincteric component of the primary tract was excised by core fistulectomy, using coagulation diathermy, and sent for histopathological appraisal. The primary tract traversing the sphincter was thoroughly curetted if granulation tissue was present, cored out if epithelialised, and chemically cleansed with dilute hydrogen peroxide followed by saline lavage. After tract preparation, patients were randomly assigned, by means of opening consecutively numbered sealed envelopes, to receiving either the collagen implant or the collagen suspended in fibrin glue.

##### **4.2.4.1 Collagen implant**

The collagen implant was cut into a strip that approximated the dimensions (width and length) of the fistula tract, so as to fit snugly within it. Once fashioned, it was drawn into position using a 0/0 nylon suture, one end of which was passed along a grooved fistula probe within the prepared tract, and the needle at the other end used to secure the implant so that it could be drawn into the tract. Excess material was trimmed at the internal and external openings, and the implant sutured into the tract at both openings, with the mucosa at the internal opening closed over the tip of the implant using 3/0 vicryl.

#### 4.2.4.2 Collagen-fibrin glue

One millilitre of the Permacol injection® was injected into a 1.5 ml sterile Eppendorf Biopur® pipette tip (Eppendorf UK Limited, Cambridge, UK), and centrifuged at 1100 rpm for 5 minutes. The saline supernatant was discarded, and the residual collagen fibres resuspended in 1.0 ml calcium chloride solution supplied with the Tisseel Kit®. The individual components of the Tisseel Kit® were then prepared as per the manufacturers' instructions. mixed, warmed in a Fibrinotherm™ (Baxter AG, Vienna, Austria) and were then drawn up into two syringes (syringe 1: fibrinogen and aprotinin; syringe 2: thrombin and collagen fibres suspended in calcium chloride solution), which were subsequently placed in a Duploject™ (Baxter AG) two-syringe clip, where they shared a common plunger. A plastic double-lumen Y-connector joined these two syringes. This apparatus was then attached to a 21-gauge cannula, passed along a grooved fistula probe, the tip of which was visualized at the internal opening. On injection, the components combined at the cannula tip to form a collagen-fibrin glue mixture. Slow withdrawal of the cannula during instillation, and visualization of the mixture extruding from both internal and external openings ensured tract filling. The collagen-fibrin glue did not run out of the fistula, but on injection, almost instantaneously, formed a clot, which was retained within the tract; this was allowed to set for 2 – 3 minutes. Excess clot from each opening was removed with scissors, and the internal opening closed with 3/0 vicryl.

In both techniques, the external opening was only partially closed, using 3/0 vicryl, so as to allow drainage of any inflammatory exudate. Gauze and mesh pants were used to protect the wound. Post-operatively, patients received regular stool softeners and bulking agents, and simple analgesics. Patients were discharged home within 24



hours of surgery. They were advised to keep the area dry for 48 hours, and to avoid swimming, cycling, horse-riding and sexual activity for 2 weeks following surgery.

#### **4.2.5 Follow-up**

At the initial 3-month follow-up, patients underwent symptom and continence assessment via a questionnaire as described in Chapter 3 (see Appendix)<sup>360</sup>, clinical examination, and repeat anal manometry and EAUS. The questionnaire also assessed the initial success of the procedure, time taken to return to work and for the perineal wound to heal, and symptoms associated with recurrence (perianal pain, swelling or discharge). Thereafter clinical follow-up was performed at 6, 9, 12, and 18 months, and then annually.

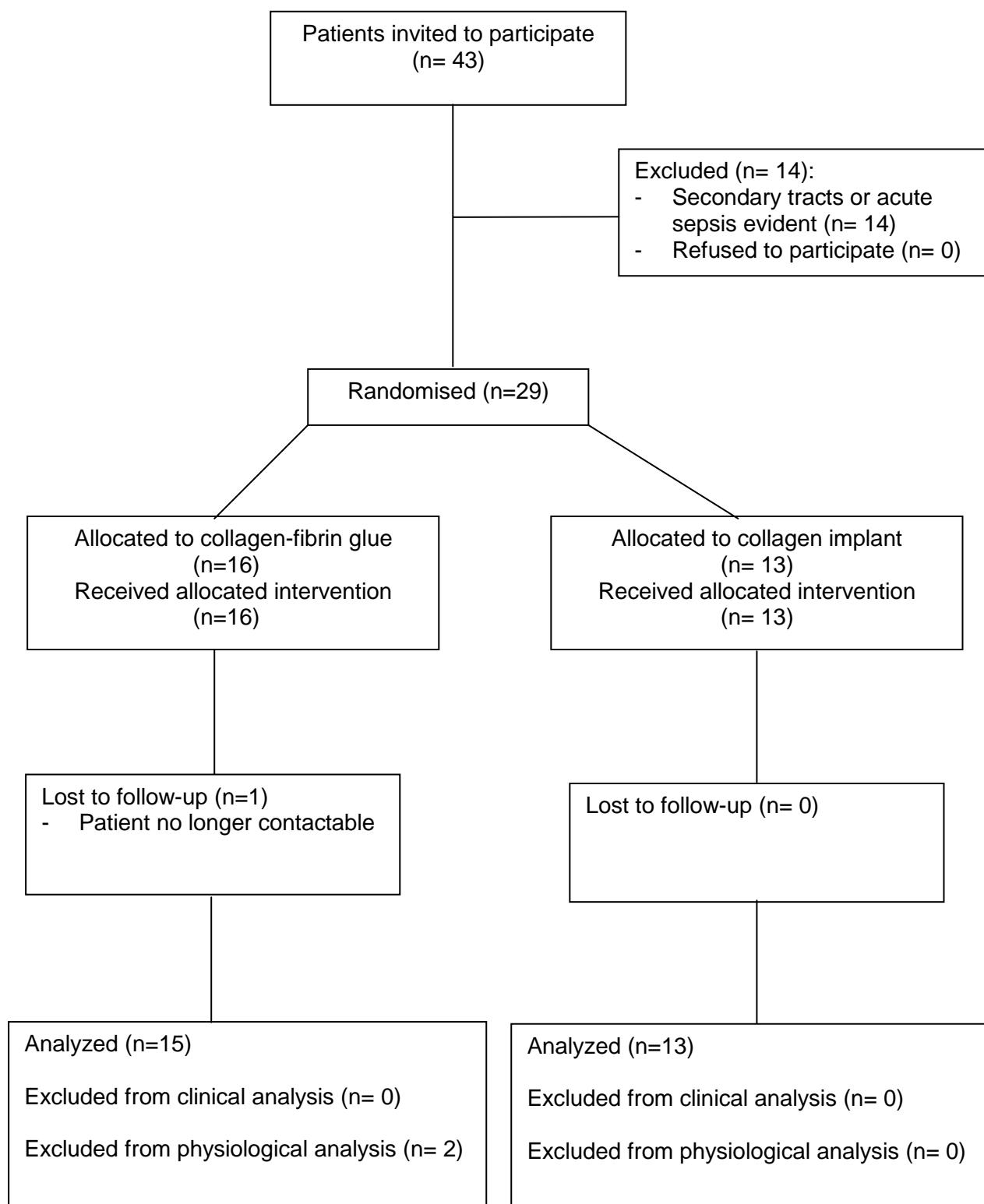
#### **4.2.6 Statistical analysis**

Comparisons were made between the fistula healing rates in the two groups (Fishers exact test), and pre and post-operative anal resting and squeeze pressures (paired t-test). Analysis was performed using a commercially available software package (Prism 4, Graftpad software, San Diego, CA, USA). Statistical significance was assigned at the 5% level.

### **4.3 Results**

The flow of patients through each stage of the trial is summarised in Figure 4.1. Forty-three patients were invited to participate from September 2004 to December 2007, of whom 29 were eligible for randomisation. All 14 ineligible patients were excluded on the basis of MRI findings of secondary tracts or abscess. Patient demographics, previous fistula surgery and fistula classification are presented in

Table 4.1. Of note, all patients had had previous fistula surgery, of whom 23 had undergone procedures to simplify the anatomy, i.e. to eradicate secondary extensions, confirmed on MRI. Sixteen patients were randomised to receive the collagen-fibrin glue, and 13 to the collagen implant. One patient in the former group was lost to follow-up, and two further patients in the same group declined postoperative physiological and ultrasound assessment. Thus, clinical data from 28, and physiological data from 26 patients were available for analysis. Histology of excised tracts revealed no features suggestive of a specific aetiology in any patient. The clinical findings are summarised in Table 4.2. No patient in either group experienced postoperative acute perineal sepsis, or continence disturbance, and sphincter integrity was unchanged. There was also no change in sphincter function. Maximum resting anal pressures and maximum squeeze increments were unaffected by surgery in either group (Table 4.3, Figure 4.2), and analysis of pressures at each station within the anal canal similarly revealed no changes in either parameter (Table 4.4). At a median of 29 (4 – 43) months, 12 of 15 (80%) patients treated with the collagen-fibrin glue were symptom free, with clinical evidence of a healed fistula, compared to 7 of 13 (54%) patients treated with the collagen implant ( $P = 0.2275$ ). Evidence of recurrence in the three patients unsuccessfully treated with the collagen-fibrin glue arose at 1, 3 and 4 months, and in the 6 failures with collagen implants, at a median of 6 months (range 1 – 13). Those patients who were symptom free reported satisfaction and those whose symptoms recurred reported dissatisfaction with the treatment.



**Figure 4.1** Flow chart of patient progression through trial

**Table 4.1** Patient demographics and fistula classification

	<b>Collagen Implant</b>	<b>Collagen-Fibrin Glue</b>
	<b>(n = 13)</b>	<b>(n = 16)</b>
<b>Median age (range)</b>	43 (35 – 53)	42 (26 – 56)
<b>Sex ratio (M : F)</b>	9 : 4	9 : 7
<b>Previous surgery:</b>		
• <i>Lay open</i>	1	2
• <i>Cutting seton</i>	1	1
• <i>Anal Fistula Plug</i>	0	1
• <i>Conversion to primary tract &amp; loose seton</i>	11	12
<b>Classification:</b>		
• <b>Intersphincteric</b>	2	2
• <b>Trans-sphincteric</b>	11	14
- <i>High</i>	0	2
- <i>Mid</i>	7	10
- <i>Low</i>	4	2

**Table 4.2** Clinical outcome

	<b>Collagen Implant (n = 13)</b>	<b>Collagen-Fibrin Glue (n = 15)</b>
<b>Wound healing time in weeks: median (range)</b>	3 (2 – 4)	4 (1 – 6)
<b>Return to work in weeks: median (range)</b>	2 (1 – 2)	1 (1 – 4)
<b>Patient satisfaction:</b>		
• <b>Very satisfied</b>	4	8
• <b>Satisfied</b>	3	4
• <b>Dissatisfied</b>	2	1
• <b>Very dissatisfied</b>	4	2
<b>Asymptomatic</b>	7	12
<b>Clinically healed</b>	<b>7</b>	<b>12</b>
• <b>Intersphincteric</b>	<b>2 / 2</b>	<b>2 / 2</b>
• <b>Trans-sphincteric</b>	<b>5 / 11</b>	<b>10 / 13</b>
- <i>High</i>	-	2 / 2
- <i>Mid</i>	3 / 7	8 / 9
- <i>Low</i>	2 / 4	0 / 2
<b>Continence disturbance:</b>		
• <b>Intersphincteric</b>	0	0
• <b>Trans-sphincteric</b>	0	0

**Table 4.3** Pre- and postoperative maximum anal pressures.

<b>Collagen-fibrin glue</b> <b>(n = 13)</b>				<b>Collagen implant</b> <b>(n = 13)</b>			
Maximum resting pressure (cmH <sub>2</sub> O)		Maximum squeeze increment (cmH <sub>2</sub> O)		Maximum resting pressure (cmH <sub>2</sub> O)		Maximum squeeze increment (cmH <sub>2</sub> O)	
pre-op	post-op	pre-op	post-op	pre-op	post-op	pre-op	post-op
<b>89 (8.2)</b>	<b>89 (7.2)</b>	<b>91 (7.9)</b>	<b>93 (7.9)</b>	<b>90 (7.7)</b>	<b>83 (4.5)</b>	<b>127 (18.1)</b>	<b>134 (18.6)</b>
<i>P</i> = 0.9459		<i>P</i> = 0.8651		<i>P</i> = 0.4022		<i>P</i> = 0.5464	

values represent: mean (SD)

**Table 4.4** Results of pre- and postoperative station pull-through manometry

distance from anal verge (cm)	Resting pressure (cmH <sub>2</sub> O)			Squeeze increment (cmH <sub>2</sub> O)		
	pre-op	post-op	<i>P</i>	pre-op	post-op	<i>P</i>
4	<b>46 (5.6)</b>	<b>39 (5.4)</b>	0.3034	<b>54 (7.0)</b>	<b>67 (9.7)</b>	0.0774
3	<b>70 (7.2)</b>	<b>61 (5.1)</b>	0.2800	<b>70 (8.8)</b>	<b>74 (9.5)</b>	0.5882
2	<b>69 (5.2)</b>	<b>70 (5.0)</b>	0.8776	<b>86 (10.4)</b>	<b>87 (9.6)</b>	0.9246
1	<b>53 (4.4)</b>	<b>56 (4.5)</b>	0.5680	<b>83 (9.0)</b>	<b>80 (9.4)</b>	0.6793

values represent: mean (SD)

results are combined (n = 26)

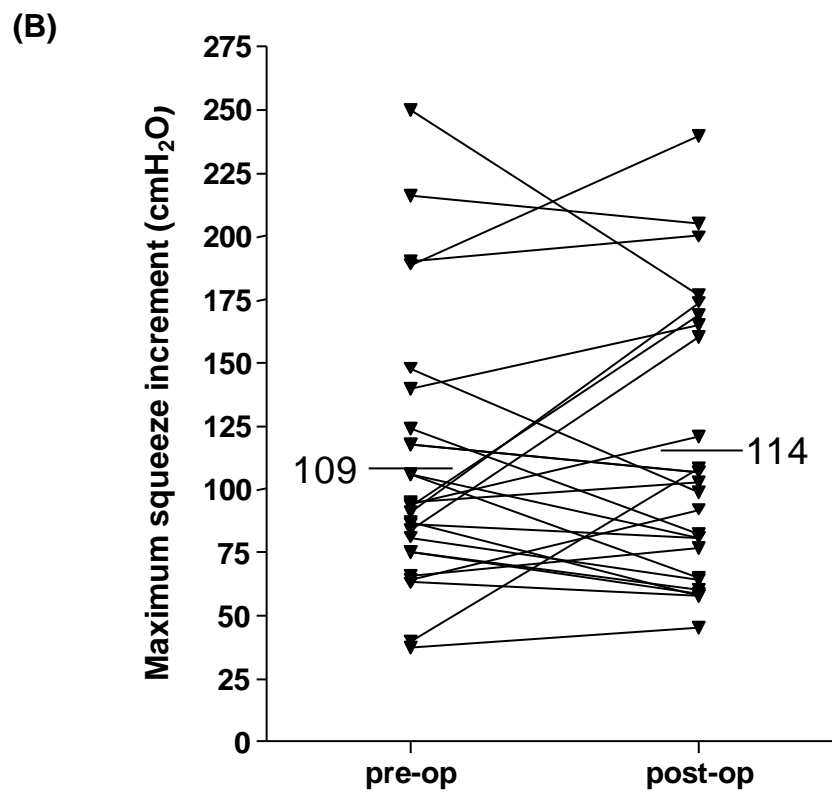
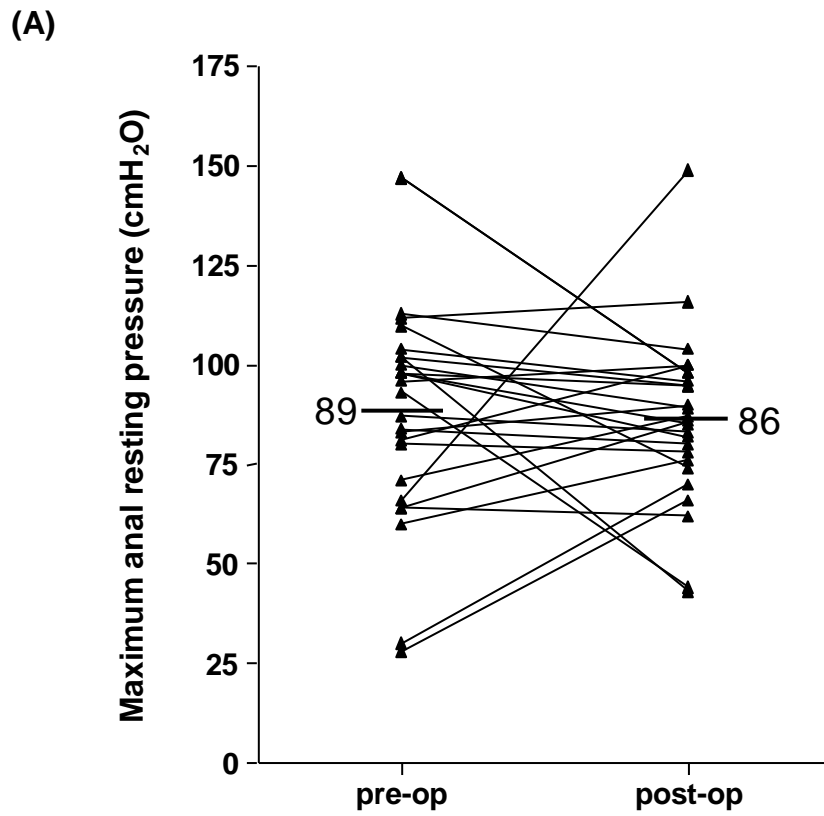


Figure 4.2 Individual changes to (A) maximum resting anal pressures and (B) maximum squeeze increments in both treatment groups. Mean values are indicated.



#### 4.4 Discussion

The results of this prospective phase 1 study to assess the role of porcine derived-cross-linked collagen (Permacol<sup>®</sup>) in the management of idiopathic anal fistulas demonstrates that the two techniques used are safe, technically feasible and potentially efficacious. At the initial 3-month follow-up, no patient in either group experienced acute perianal sepsis, symptoms of incontinence, or changes to anal sphincter function or integrity. Technically, the procedures were not difficult to perform, and should not impact on any further treatment in the event of failure. Indeed, two patients in whom initial treatment failed (one from each arm of the trial) have subsequently undergone collagen-fibrin glue treatment (outside the trial) with healing to date. Both techniques have the potential to permanently heal idiopathic anal fistulas: at a median of 2½ years, 80% of patients treated with the collagen-fibrin glue, and 54% of patients (P = NS) treated with the collagen implant were healed. Patient satisfaction was understandably linked to the successful eradication of their fistula and related symptoms. As this was primarily a proof of concept study, a third (control) arm was not included, and pre-study statistical considerations such as sample size calculations were not performed. It is acknowledged therefore that failure to demonstrate superiority of one technique over the other may have been due to the relatively small number of patients studied.

It can be argued that patients should have undergone post-operative MRI to provide radiological confirmation of fistula healing over external opening epithelialisation. However, the evidence to date indicates that where skin healing is mistaken for evidence of actual fistula healing, almost all recurrences occur within 16-months<sup>252</sup>. Additionally, it is unlikely that standard anal fistula MRI protocols are of sufficient sensitivity to differentiate between a persistent fistulous tract, a healing tract with collagen in situ, or the scarred remnant of a healed fistula. An alternative sequencing

technique aimed at predicting fistula healing has been described<sup>252</sup>, and may be of future use in this setting.

It is likely that the initial key to the success of all sphincter preservation techniques, regardless of whether they are purely 'surgical' or employ a biological or synthetic material, is in providing the correct environment for healing to occur. Before the advent of biomaterial use in fistula surgery, the results of advanced techniques aimed at fistula eradication without sphincter compromise, notably advancement flaps, indicated that eradication of secondary extensions and abscesses were a prerequisite for success. Indeed, the presence of such complicating factors meant that the numbers of patients eligible for entry into this trial (n = 29) was lower than the number initially recruited (n = 43). The high prevalence of complex fistulas reflects the predominantly tertiary referral nature of the practice, with all 29 patients having previously undergone some form of fistula surgery, twenty-three of whom had undergone surgical treatment specifically to simplify fistula anatomy, subsequently confirmed on MRI.

On theoretical grounds, attention to the primary tract is also necessary to optimise the chances of success by biomaterial track plugging. The aim is to convert the chronically inflamed or epithelialised tract, often surrounded by dense fibrosis, to an acute wound, thereby allowing the healing cascade to recommence with the potential for progression to complete tissue repair<sup>253</sup>. In the present study this was achieved through core out of the extrasphincteric component, and either de-epithelialisation or thorough curettage, followed by chemical cleansing, of the sphincteric component. Failure to adequately remove all granulation or epithelial tissue lining the fistulous tract affects fibroblast and endothelial cell migration, and possibly in conjunction with incomplete removal of the presumed source (the diseased intersphincteric anal gland), will inhibit healing<sup>252;253;374</sup>. Nonetheless, if adequate tract preparation alone

was sufficient to guarantee permanent fistula healing, it could be argued that those sphincter preservation techniques in which this is performed, such as fistulectomy, with or without closure of the internal opening (by stitch closure or mucosal advancement flaps) should be more successful than the evidence suggests<sup>268;297;298;375</sup>.

An additional success limiting factor has therefore been proposed, that fistula persistence/ recurrence may be due to the lumen of the tract remaining as a void, and the lack of contact of apposing prepared walls thus preventing cellular interaction and void-filling tissue growth<sup>374</sup>. Fistulotomy is the most successful fistula eradication strategy to date, and the most likely reason is that by laying open the tract all the aforementioned aims are achieved in conjunction with the conversion of an enclosed void to an open wound. Hence, for any sphincter preserving techniques to achieve equivalent efficacy, following creation of an appropriate environment for fistula repair, an infill material is required, which not only bridges the defect but intuitively allows full host tissue incorporation and neovascularisation, whilst withstanding premature degradation and bacterial colonisation.

Fibrin glue, the most widely studied biomaterial in the treatment of anal fistulas, is associated with recurrence rates up to a 100% at long-term follow-up<sup>263;306-310</sup>. A number of authors have suggested its failure is secondary to: a liquid consistency, allowing it to run out of the fistula tract; the inability of fibrin glue to securely close the internal opening; and extrusion of the glue shortly after surgery because of raised intra-anal pressures<sup>307;310</sup>. However these concerns are not supported by the literature, or the personal experience of this technique by both the author and supervisor of this thesis<sup>318</sup>. The effect of excluding patients with sepsis and secondary extensions, and employing techniques to optimise the primary tract is not clear. Some studies have excluded patients with sepsis beyond the primary track

alone, many have not, and with numerous other variables in relation to management, it is impossible to determine their specific effect upon outcome<sup>307</sup>. Additionally, studies have shown that fibrin glue does not exhibit those specific biological requirements which are most likely to ensure fistula healing, namely host cell integration, neovascularisation<sup>323;324</sup>, and resistance to early degradation<sup>325</sup>.

The anal fistula plug (AFP) technique, first published in 2006, was developed in order to address both the biological and perceived mechanical concerns associated with fibrin glue. Mechanically, the plug was solid, and therefore could not run out of the tract on insertion, and could be sutured within the fistula theoretically avoiding early extrusion<sup>333</sup>. Biologically, the material from which the plug was fashioned, porcine small intestinal mucosa, had been shown in both laboratory and clinical trials to be capable of supporting host soft tissue repair<sup>18;128</sup>, and certain studies have further demonstrated its resistance to infection and subsequent enzymatic breakdown<sup>77;170</sup>. This first report showed a significant improvement in early healing rates for high fistulas treated with the AFP compared to those treated with fibrin glue (87% *versus* 40%,  $P < 0.05$ ). However subsequent reports have revealed a similar range of healing (24 – 93%) to that associated with fibrin glue (see Table 1.8); and this is despite a published consensus, by surgeons experienced with procedure, on the proper technique (secondary tracts are not eliminated, and debridement or curettage of the primary tract is not advised as this is thought to increase its size and therefore the risk of plug expulsion), patient and fistula selection criteria, and pre- and postoperative management<sup>335</sup>. The majority of failures occur in the first 3-months following surgery, and ironically up to 40% of these are due to early plug extrusion<sup>336</sup>. No published theory has been proposed as to the cause of the remaining failures, although certain reports have shown that untreated secondary extensions are not significantly associated with fistula recurrence<sup>334;340;341;344</sup>.

However whilst an unprepared fistula tract does not seem to prevent plug extrusion, it does (as previously discussed) prevent fibroblast and endothelial cell migration which is crucial for healing. Additionally, although Surgisis<sup>®</sup> (SIS) is capable of supporting soft tissue repair in clean wounds, whether it is the best scaffold for repair in a clean-contaminated or contaminated field is unclear from the available literature. In a canine study, SIS was reported as being relatively resistant to persistent infection, following deliberate bacterial contamination, and to support constructive tissue remodelling<sup>166</sup>; whereas in a murine model, SIS was found to serve as a nidus for microbial attachment and growth, thus exacerbating surgical site infection<sup>372</sup>. In clinical ventral hernia studies, some authors have concluded that the use of SIS in contaminated or potentially contaminated fields is safe, feasible and that, on later examination, in most cases the implant becomes totally integrated into the host<sup>77</sup>; others have reported accelerated degradation when SIS has been used to reconstruct abdominal wall defects in contaminated environments, which the authors hypothesized was responsible for early hernia recurrence<sup>169;170</sup>.

Permacol<sup>®</sup> is a porcine-derived, acellular, isocyanate cross-linked, dermal collagen matrix, both preparations of which (a solid sheet and milled fibres in suspension) have been demonstrated *in vitro* and in animal model studies to be biocompatible constructs which, unlike fibrin glue and Surgisis<sup>®</sup>, support cellular integration and ECM deposition whilst resisting premature enzymatic degradation, respectively<sup>180;182;183;186;374;376</sup>. The differences in their morphology offer distinct individual advantages for fistula repair. The solid implant can be easily sited and fashioned to the dimensions of a fistula, in a similar manner to the anal fistula plug, although (as shown from the work in Chapter 2, and in conjunction with other laboratory studies) full thickness cellular integration can be prolonged<sup>182;183;186;377</sup>.

The milled fibre preparation needs to be retained within the fistula, achieved in this

study by its suspension in fibrin glue, which through its more liquid nature at instillation enables more complete filling of the track. Following fibrin glue induced epithelialisation of the tract openings and glue resorption, the remaining collagen fibres can provide a robust network of bioscaffolds, with the spaces between potentially allowing more rapid full thickness cellular integration<sup>374;376</sup>.

The main aim of this study was to establish whether a role existed for a xenogeneic cross-linked collagen (Permacol<sup>®</sup>) in the management of idiopathic anal fistulas, and secondarily whether efficacy was influenced by the physical format. Currently, greater numbers with longer follow up are required to answer these questions, and thus the study remains on going. If sufficient long term efficacy can be demonstrated, and one format proven over the other, then an appropriately powered randomised control trial would need to be constructed, although the choice of a suitable control (such as rectal mucosal or anodermal advancement flap, fistulectomy with direct closure, or fistulotomy with immediate sphincter repair) in a field in which there is no gold standard, and a wide-range of techniques, each with its proponents, presents a challenge in itself. In the interim, efforts must continue to explore biological agents, both in respect of the agents themselves, and the optimum conditions for their use in the management of an often challenging condition.

# Chapter 5

Discussion of thesis &  
Proposals for future work

This thesis has explored the role of biomaterials in the management of parastomal hernia and anal fistula, and identified areas where either adaptation of an existing technique, the cutting seton for anal fistulas, or use of a new biological material, xenogeneic cross-linked collagen (Permacol<sup>®</sup>), for prevention of parastomal hernia and treatment of idiopathic anal fistula can be utilised to potentially improve the clinical outcome for those patients afflicted by these chronic conditions. Both diseases essentially represent a failure to progress along the wound healing pathway, and their management should therefore involve strategies which address those specific areas in which healing has been impeded.

Parastomal hernias, and indeed all abdominal wall hernias, are at least in part considered to represent the end-point of a condition in which there is a shift of the collagen ratio from type I collagen, that predominantly confers tensile strength, to “immature” type III collagen, a temporary scaffold for fibroblast attachment<sup>50</sup>. The weakened abdominal wall is consequently prone to herniation secondary to any variety of situations or conditions that increase the intra-abdominal pressure. Any material used to reinforce or repair the abdominal wall should intuitively therefore either be predominantly composed of type I collagen or correct the balance of collagen metabolism. The logic behind the prophylactic placement of mesh to prevent parastomal herniation has been previously described, and its success well documented<sup>34;98;123;125;127</sup>. However, prior to the commencement of this thesis only the use of synthetic mesh had been described, which is associated with a chronic inflammatory foreign body response and therefore the potential for tissue fibrosis, bowel erosion, and increased susceptibility to sepsis. The role of Permacol<sup>®</sup>, a more biocompatible material composed of predominantly type 1 collagen, was investigated in Chapter 2. The aim of that phase 1 study was to assess the safety, feasibility and potential efficacy of using this implant to prevent parastomal hernias, and to evaluate



the human host response to its presence. Twenty-five patients were prospectively recruited, of whom 15 were randomised to a defunctioning stoma with the implant and 10 to a conventional stoma. Follow-up included regular symptom questionnaires, clinical examination, stoma site ultrasound, and serum inflammatory markers. At a median of 9.5 months a parastomal hernia was clinically evident in 3 of 10 patients without the implant, and in 1 of 15 patients with the implant. The latter patient prompted a change in the study methodology, in that the implant was subsequently sited in the pre-peritoneal opposed to fascial onlay position. There were no other associated clinical complications, ultrasound evidence of chronic seroma formation or serological evidence of a systemic inflammatory response. Histological data from 90% of the patients who received the implant and underwent stoma reversal showed that all host responses were limited to the periphery of the implant and native pores. These included a minimal inflammatory response and implant degradation, evidence of fibrovascular infiltration and MMP-1 activity, and organized deposition of host collagen, fibronectin and laminin. The specific areas of trial methodology that could have been improved upon have been discussed in the aforementioned chapter, but nonetheless the data demonstrated that this particular cross-linked xenogeneic collagen implant is biocompatible and resistant to degradation in most patients, and that although fibrovascular in-growth and ECM deposition were limited, the implant has excellent potential for soft tissue reinforcement. It is therefore safe, technically feasible to use in this setting, and has the potential to prevent parastomal herniation. Following on from this work a multi-centre randomized controlled trial comparing Permacol<sup>®</sup> reinforcement of permanent stomas versus both standard stoma formation and reinforcement with a light-weight polypropylene mesh has been designed, and addresses both clinical outcome measures and cost benefits. Ethical approval has been obtained and patient recruitment is in progress. Future work in

this arena initially needs to focus on whether all patients required to have a stoma should undergo prophylactic primary mesh placement or if the procedure should be targeted at those most at risk of such a complication. Ultimately, however research needs to address abdominal wall hernias in general, and in this regard there seem to be two areas of interest. One involves identifying those factors (genetic and environmental) responsible for pathologically shifting the pattern of collagen deposition, with the aim being to employ measures which rectify this imbalance. These could include implants that contain locally acting constituents, or pharmaceutical agents that act systemically, to affect fibroblast proliferation and collagen metabolism<sup>50;356</sup>. An alternative strategy, rather than focusing on improving the quality of scar tissue, is to evaluate the role of skeletal muscle regeneration thereby restoring the native abdominal wall to its pre-pathological mechanical state<sup>378;379</sup>.

Anal fistulas are characterised as being in a state of chronic inflammation and fibroblast induced granulation, with an inability of the ECM to progress to full tissue reconstitution. Fistulotomy is the most successful treatment to date presumably as it converts a chronic enclosed to an acute open wound and eradicates any causative and perpetuating pathological factors, which is generally accepted but not absolutely proven to be the diseased intersphincteric anal gland. As previously discussed, the variable amount of anal sphincter complex enclosed by the fistula means this strategy is not suitable for all patients, and techniques for managing such patients have been divided into those which traditionally attempt to minimise sphincter damage and functional outcome, and more recent treatments which attempt to preserve these completely. The cutting seton technique lies within the former group. Analysis of published results of this technique show that variations in the speed of

tissue severance have little effect on fistula eradication rates but impact considerably upon continence rates: the more rapidly the sphincter is divided the greater the degree of incontinence. This is hardly surprising when one considers that myofibroblast induced wound contraction and fibrosis only start to feature in the wound healing pathway 5-7 days following injury, tends to peak at two-weeks, and can persist for many more<sup>6</sup>. Additionally, the cutting seton technique is associated with pain and the need for repeat tightenings. Chapter 3 retrospectively assesses the short and intermediate outcomes of a modification of this technique, using a 'snug' silastic seton, to treat idiopathic anal fistulas by dividing the enclosed tissue in a slower more comfortable fashion. Twenty-nine patients' notes were reviewed for short-term analysis, of whom 16 participated in a medium-term review at a median of 42 months. The seton spontaneously cut out in 15 out of 29 (52%) fistulas after a median of 24 weeks, and the remainder required division of seton enclosed residual tissue (< 5 mm) at a median of 35 weeks. All the patients' fistulas healed, but 34% had minor continence disturbance in the short-term, and in 25% incontinence persisted into the medium-term. Interestingly, despite these levels of incontinence all the patients were at least 'satisfied' with their outcome, which concurs with other studies that show that patients are prepared to accept minor degrees of functional impairment in preference to the discomfort associated with chronic anal sepsis<sup>250;360</sup>. Nonetheless complete preservation of continence whilst maintaining the high levels of fistula eradication described remains the primary goal of fistula surgeons. Attempts to achieve these aims by employing biological materials, such as fibrin glue and porcine small intestinal submucosa (Anal Fistula Plug™ SIS®), were based on sound principles but have demonstrated widely variable fistula eradication rates, although in general continence has not unsurprisingly been affected. Technical factors, such as early implant extrusion, have been cited as one of the more

consistent reasons for their failure, and although this appears to be the case in the early post-operative period, it does not account for those whose treatment failed despite the material remaining in situ. Two other factors are most likely responsible for treatment failure in this latter group of patients. The environment into which the materials were introduced does not seem to have been optimised to facilitate healing, and the materials themselves may lack one or more of the requirements for successful fistula repair. These include full host tissue incorporation and neovascularisation, withstanding premature degradation and avoiding bacterial colonisation. *In vitro* and animal model studies, and the human histological data presented in Chapter 2, indicate that Permacol<sup>®</sup> can mostly fulfil these criteria.

A prospective phase 1 study to assess the potential role of Permacol<sup>®</sup> in the management of idiopathic anal fistulas was therefore constructed. On account of concerns regarding the ability of this implant to allow full host integration, two trial arms were incorporated in to this feasibility study. One involved suturing a strip of the implant (from the sheet format described in Chapter 2) within the fistula tract. The other involved using milled Permacol<sup>®</sup> fibres suspended in fibrin glue and thence injecting them into the fistula. The premise being that the fibrin glue would retain the collagen fibres within the tract, and that those qualities which most likely contributed to the failure of the glue as fistula repair material could be used advantageously. The fibrin clot would induce epithelialisation at the fistula openings and then rapidly degrade, allowing the individual collagen fibres to act as bridging scaffolds for fibroblast attachment and collagen synthesis, and eventually becoming part of the developing ECM. Additionally, the spaces between the fibres would potentially allow more rapid cellular integration and neovascularisation than the Permacol<sup>®</sup> sheet. Patients, unsuitable or unwilling to undergo fistulotomy were recruited. Pre-operatively participants underwent symptom, continence and anal physiology

assessments, and magnetic resonance imaging. Patients with secondary extensions or acute sepsis were excluded. At operation, after removal of any granulation or epithelial tissue lining the fistula tract, participants were randomised to receiving one of the aforementioned collagen formats. Follow up included repeat symptom, continence and physiological assessments at 3 months, and thence regular clinical review. Twenty-nine of 43 entrants were eligible for inclusion. Thirteen patients received the collagen implant, and 16 collagen-fibrin glue. Three months post-operation no patient experienced acute sepsis or continence disturbance, and sphincter function and integrity were unchanged. At 29 months, 12 of 15 (1 lost to follow-up) patients treated with collagen-fibrin glue were healed, compared to 7 of 13 who received the implant. Therefore in the short to medium term, this study demonstrated that the two techniques using xenogeneic cross-linked collagen were safe, in terms of both avoiding acute sepsis and damaging the anal sphincter, technically feasible and had the potential to heal fistulas. There was a trend in favour of the fibre suspension although larger patient numbers and longer follow up are required to prove which of these techniques is superior. The difficulties of then comparing the more efficacious of the techniques to more established methods of fistula eradication in a field dominated by personal preferences, variable pre-operative degrees of anal continence, and low levels of evidence has been previously discussed. An alternative may be to perform a multi-centre study comparing the technique under trial with that of the participating surgeons' preferred technique. There are a number of additional areas that will also need to be addressed in the future, and these include identifying why fistulas persist following an episode of acute sepsis and the possible role of any hitherto unrecognised pathogens (such as *Helicobacter pylori* in the pathogenesis of peptic ulcer disease); why males are more prone to fistulas than females and the possibility of differences

in anal gland sensitivity to androgens<sup>199</sup>; and to examine the local milieu required to facilitate tissue repair. The development of biomaterials specifically designed to combat any underlying pathological factors would also represent a great leap forward. Concepts include biomaterials impregnated with constituents for local hormonal or ECM manipulation, and in the case of cross-linked materials, reduced degrees of cross-linkage to allow for better tissue integration whilst still withstanding premature degradation. Lastly, the success of negative pressure vacuum assisted dressings for the management of chronic wounds<sup>380;381</sup>, and more recently rectal anastomotic dehiscence<sup>382;383</sup>, suggests a possible role for their use in the management of anal fistulas.

In conclusion, this thesis has presented a number of novel applications for both traditional and contemporary biomaterials in the management of parastomal hernia and anal fistula, and although disparate pathologies were addressed, both they and the thesis were unified by demonstrating that an understanding of the specific disease pathology, wound healing, and the host response to materials (synthetic and biological) are central to their successful management.

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## Appendix

### Follow-up questionnaire for fistula recurrence, postoperative anal incontinence, and quality of life.

1. Name:
2. Date of operation:
3. Did the operation heal the fistula Yes or No  
(Please ring correct answer)
4. How long did it take for the fistula wound to heal after the operation? \_\_\_weeks
5. How long after your operation did you return to work? \_\_\_weeks
6. Since your operation have you experienced any pain in the region of your anal fistula, similar in nature to the pain you were experiencing before your operation  
(Please ring correct answer) Yes or No
7. Since your operation have you experienced any discharge (fluid/ pus) from your anal fistula?  
Never   
For a short period of time after my operation   
Ever since my operation
8. Since your operation have you noticed any swelling in the region of your anal fistula?  
(Please ring correct answer) Yes or No
9. Since your operation have you had any difficulty distinguishing between gas and stool?  
A. Never   
B. For a short period of time after my operation   
C. Ever since my operation   
  
Rarely (less than once a month)   
Sometimes (more than once a month)   
Frequently (more than once a week)

10. Since your operation have you had any problem holding gas?
- A. Never
  - B. For a short period of time after my operation
  - C. Ever since my operation
  - Rarely (less than once a month)
  - Sometimes (more than once a month)
  - Frequently (more than once a week)

11. Since your operation have you had any problem with soiling of your underwear?
- A. Never
  - B. For a short period of time after my operation
  - C. Ever since my operation
  - Rarely (less than once a month)
  - Sometimes (more than once a month)
  - Frequently (more than once a week)

12. Since you operation have you had accidental bowel movements?
- A. Never
  - B. For a short period of time after my operation
  - C. Ever since my operation
  - Rarely (less than once a month)
  - Sometimes (more than once a month)
  - Frequently (more than once a week)

13. Do you have to wear a pad?
- Never
  - Only at night
  - Sometimes (Daytime)
  - All the time (Daytime)

14. Are you satisfied with the results of the operation?
- Very satisfied
  - Satisfied
  - Dissatisfied
  - Very dissatisfied

15. Does this problem affect your lifestyle? (Mark the best answer for each column)

	Physical Activities	Social Activities	Sexual Activities
Not at all			
To some extent			
Greatly			

16. Have you experienced any other symptoms/ problems since your operation  
(please list):



## Summary of relevant publications

1. Hammond TM, Porrett TRC, Scott SM, Williams NS, Lunniss PJ. Management of idiopathic anal fistula using cross-linked collagen: a prospective phase 1 study. Accepted by *Colorectal Disease*, July 2009.
2. Hammond TM, Huang A, Prosser K, Frye JN, Williams NS. Parastomal hernia prevention using a novel collagen implant. A randomised controlled phase 1 study. *Hernia*. 2008; 12: 475-81
3. Hammond TM, Chin-Aleong J, Navsaria H, Williams NS. Human *in vivo* cellular response to a cross-linked acellular collagen implant. *Br J Surg*. 2008; 95: 438-46
4. Hammond TM, Lunniss PJ. Principles and contemporary management of anal fistulas. *Gastrointestinal Nursing* 2007; 5 (2): 32-40 (Invited review)
5. Hammond TM, Lunniss PJ. Novel biomaterials in the management of anal fistulas. *Dis Colon Rectum* 2006; 49: 1463-4 (Letter to the Editor)
6. Hammond TM, Knowles CH, Porrett T, Lunniss PJ. The Snug Seton. Short and medium term results of slow fistulotomy for idiopathic anal fistulae. *Colorectal Dis*. 2006; 8: 328-37.
7. Hammond TM, Lunniss PJ, Grahn MF. Fibrin glue in the management of anal fistulae. *Colorectal Dis*. 2004; 6: 308-19.  
  
Accepted onto the Database of Abstracts of Reviews of Effects (DARE), Centre for Reviews and Disseminations, July 2006.

## Summary of relevant presentations

1. Novel treatments for idiopathic anal fistulae. Hammond TM, Porrett TRC, Scott SM, Williams NS, Lunniss PJ. Royal Society of Medicine, Coloproctology Section Overseas Meeting, Paris, May 2007. Awarded a Travelling Fellowship.
2. Parastomal hernia prevention using a novel collagen implant: a prospective randomised pilot study. Hammond TM, Huang A, Frye J, Prosser K, Williams NS. Winner of Colon & Rectal Disease Research Foundation Prize, Association of Coloproctology of Great Britain and Ireland, Gateshead, July 2006.
3. Collagen seton versus collagen-fibrin glue mixture in the management of idiopathic anal fistulae: a prospective randomised pilot study. Hammond TM, Porrett T, Scott SM, Williams NS, Lunniss PJ. Association of Coloproctology of Great Britain and Ireland, Gateshead, July 2006.
4. Parastomal hernia prevention using a novel collagen implant: a prospective randomised pilot study. Hammond TM, Huang A, Frye J, Prosser K, Williams NS. Six of the best general short papers session, Association of Surgeons of Great Britain and Ireland, Edinburgh, May 2006.
5. Human *in vivo* response to a cross-linked collagen implant. Hammond TM, Chin-Aleong J, Navsaria H, Williams NS. Society of Academic & Research Surgery (SARS), Edinburgh, January 2006.
6. The Snug Seton: Short and medium term results of slow fistulotomy for idiopathic anal fistulae. Hammond TM, Knowles CH, Porrett T, Lunniss PJ. Tripartite Colorectal Meeting, Dublin, July 2005.
7. The contemporary management of anal fistulae. Hammond TM, Lunniss PJ. Invited presentation at the Annual Meeting of the Hungarian Association of Coloproctologists, April 2005.