A study into the prevention of parastomal herniation

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Declaration of my contribution

I, Alexander Hotouras, confirm that all studies presented within this thesis have been my own work. The thesis, was registered in September 2011, and submitted in December 2013. All surgery on patients at The Royal London Hospital and other NHS institutions was performed by Consultant Surgeons working at these institutions. I attended all surgical procedures on all patients where possible. The real time PCR experiments detailed at Chapter 5 were performed by Dr Evonne Chin-Smith. I confirm that where information has been derived from other sources, this has been indicated in the thesis.

Abstract

A hernia frequently complicates abdominal stoma formation. The aetiology of parastomal herniation is claimed to be multi-factorial but currently only age and trephine diameter have been shown to independently predict its development. Open or laparoscopic repair of a symptomatic parastomal hernia is frequently challenging and is associated with unsatisfactory recurrence rates. As a result, many affected patients are managed non-operatively.

Prevention of parastomal herniation by prophylactic mesh reinforcement of the stoma site is a new strategy that may reduce its incidence. Manual mesh implantation, however, is thought to increase the operating time and is considered cumbersome, particularly in laparoscopic surgery. As a result, routine reinforcement of the stoma site is not currently standard practice within the National Health Service. Thus, there is a need for a simple and quick technique for stoma formation which avoids creating an oversized defect and simultaneously reinforces the trephine with mesh.

The aims of this thesis included: (i) understanding the aetiopathogenesis of parastomal herniation, assessing its impact on patients' quality of life and examining the outcomes associated with current therapeutic strategies in order to find novel therapies that may lead to its prevention; (ii) assessing the safety, reproducibility and efficacy of the **S**tapled **M**esh stom**A** **R**einforcement **T**echnique (SMART) in preventing parastomal herniation and (iii) investigating the contribution of the rectus abdominis muscle to the development of herniation.

A detailed literature review of PubMed and Medline databases confirmed that stoma formation through the rectus muscle is complicated by parastomal herniation in 50%-80% of

cases. Surgeons have underestimated its impact on patients' quality of life. There is no conclusive evidence that alternative techniques (e.g. extraperitoneal, lateral rectus abdominis positioned stoma) are superior. Open and laparoscopic parastomal hernia repair have similar recurrence rates up to 50%. Prophylactic reinforcement of the stoma trephine with mesh in the sublay or subperitoneal position is safe and appears to reduce the herniation rate but it is difficult laparoscopically and does not address the issue of trephine size when a defect <25mm is associated with a reduced herniation risk.

The **S**tapled **M**esh stom**A** **R**einforcement Technique (SMART) obviates the technical issues associated with routine stoma formation and reinforcement. In a pilot study with patients at high risk for herniation, SMART was found to be safe and reproducible and reduced the herniation rate to 18%. Preliminary results of the international multicentre randomised controlled trial in all patients undergoing permanent stoma formation show that SMART reduces the herniation rate compared to the standard technique, without added morbidity and minimal impact on the operating time.

A radiological study assessing the contribution of the rectus abdominis muscle into the development of parastomal herniation showed that the abdominal musculature undergoes postoperative changes consistent with atrophy with postoperative muscle density being higher in patients without parastomal herniation.

In conclusion, at this moment in time, prophylactic mesh reinforcement should be offered to all patients undergoing elective permanent stoma formation. The SMART procedure has the potential to change current surgical practice. The contribution of the rectus muscle to the development of herniation warrants further research since improving muscle repair and regeneration may result in therapeutic benefits.

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Publications and presentations derived from this thesis

Original Publications

1. Hotouras A, Murphy J, Thaha M, Chan CL. The persistent challenge of parastomal herniation: a review of the literature and future developments. *Colorectal Dis*. 2013; 15(5):e202-14
2. Hotouras A, Murphy J, Power N, Williams NS, Chan CL. Radiological incidence of parastomal herniation in cancer patients with permanent colostomy: what is the ideal size of the surgical aperture? *Int. J. Surg*. 2013; 11(5):425-7.

Oral Presentations

1. Hotouras A, Parsai A and Chan CL. Improving abdominal muscle thickness and density: Is this a potential therapeutic target for the prevention of parastomal herniation? *Royal Society of Medicine, Section of Coloproctology*, November 2013.
2. Hotouras A. Research Update on PROPHECI and SMART trials. *National IA Meeting*, Bristol, 2013
3. Hotouras A, Chan CL and Williams NS. The "SMART" randomised controlled trial. *European Society of Coloproctology (ESCP) Congress*, Copenhagen, 2011.
4. Hotouras A, Chan CL and Williams NS. The SMART technique for the prevention of parastomal herniation. *German Colorectal Congress*, Munich, 2012.

5. Bryant C, Hotouras A, Thaha MA, Chan CL and Williams NS. The use of cadaveric workshops to disseminate novel surgical techniques among colorectal surgeons. *Association of Surgeons of Great Britain and Ireland (ASGBI) International Congress*, Liverpool, 2012.

6. Hotouras A. The prevention of parastomal herniation: Are you a 'SMART' surgeon? *13th International Colorectal Forum*, Verbier, 2012

7. Hotouras A, Williams NS, Bhan C, Thaha MA and Chan CL. Early Results of the Stapled Mesh Stoma Reinforcement Technique (SMART). *ASGBI International Congress*, Bournemouth, 2011.

8. Hotouras A, Williams NS, Berg M and Chan CLH. The SMART technique for the prevention of parastomal herniation: A video demonstration. *ASGBI International Congress*, John Wiley Session, Bournemouth, 2011

9. Hotouras A, Thaha M.A, Chan C.L.H, Williams N.S. A randomised controlled trial of **S**tapled **M**esh stom**A** **R**einforcement **T**echnique (SMART) versus standard technique to assess effect on parastomal herniation. *ASCPGBI International conference*, Birmingham, 2011 (presentation in trials section, selected in open competition).

10. Hotouras A, Williams NS, Bhan C, Thaha M.A and Chan CLH. The SMART technique for the prevention of parastomal herniation: A video demonstration. *ASCPGBI International Conference*, Birmingham, 2011.

Poster Presentations

1. Hotouras A, Al-Jilaihawi S, Foster C, McDowell S, Hard H and Chan CL.Stoma related morbidity in infants operated for Hirsprung's disease: A 17-year single centre experience. *European Society of Coloproctology (ESCP) Congress*, Vienna, 2012.
2. Mozaffari M, Al-Jilaihawi S, Hotouras A, *et al*: What is the incidence of parastomal herniation in paediatric patients? *XXV International Symposium on paediatric surgical research*. London, 2012.
3. Hotouras A, Thaha MA, Power N, Chan CL and Williams NS. Preventing Parastomal Hernias: How critical is the trephine size. *European Society of Coloproctology Congress, Copenhagen*, 2011.
4. Hotouras A, Thaha MA, Power N, Chan CL and Williams NS. Preventing Parastomal Hernias: How critical is the trephine size. *ASCPGBI, Birmingham*, 2011.

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CHAPTER 1

Background to thesis and review of the literature

"Some degree of herniation around a stoma is so common that this complication may be regarded as inevitable "

Professor John Goligher, 1984

1.1 Introduction

An abdominal stoma (Greek for *mouth*) is a surgically created opening in the abdominal wall that allows exteriorisation of the gastrointestinal or urinary tract. Stomas may therefore be classified according to the organ they involve (i.e. gastrostomy, jejunostomy, ileostomy, colostomy or urostomy). Gastrointestinal stomas may be temporary or permanent. Temporary stomas are usually fashioned for feeding (e.g. gastrostomy, jejunostomy), to divert the faecal stream from a diseased bowel segment (e.g. loop ileostomy or colostomy) or to "protect" a gastrointestinal anastomosis prior to healing (e.g. defunctioning loop ileostomy covering a low rectal anastomosis). A permanent stoma (e.g. end ileostomy or colostomy) is created when restoration of gastrointestinal continuity is not technically feasible, carries a high risk for the patient or is associated with unacceptable functional outcome.

Approximately 102,000 people are living with an abdominal stoma in the United Kingdom and around 20,000 new stomas are fashioned annually, of which 50% are permanent.[1 2]The most common complication of permanent stoma is parastomal herniation which may impact adversely not only on patients' quality of life and psychological well-being but also on healthcare resources.[3-5]

The work in this thesis is concerned with herniation complicating permanent ileostomies and colostomies as temporary stomas are usually reversed within a period of six months with closure of the abdominal wall defect.

1.2 Abdominal herniation and theories of development

A hernia is the protrusion of an organ or the fascia of an organ through the wall of the cavity that normally contains it.[6] The abdominal cavity is the most common site for herniation with 20 million such hernias repaired worldwide every year.[6] Abdominal hernias develop through a hole or "defect" via which adipose tissue or abdominal organs covered by peritoneum may protrude. Conditions (e.g. obesity, pregnancy, ascites, chronic cough) that raise the intra-abdominal pressure are thought to stretch or weaken the abdominal muscles and contribute to the development of such hernias. Examples of abdominal herniation include:

(i) inguinal hernias (70-80% of all abdominal hernias) in which the inguinal canal is entered via a congenital weakness at the internal inguinal ring (indirect hernia) or an acquired weakness in the posterior wall (direct henia)

(ii) umbilical/paraumbilical hernias (5-15% of abdominal hernias) which involve protrusion of intra-abdominal contents through a "defect" at the site of passage of the umbilical cord through the abdominal wall

(iii) epigastric hernias (4-7% of abdominal hernias) that occur between the umbilicus and the xiphisternum in the midline

(iv) femoral hernias (4-6%) occur below the inguinal ligament and are carry a greater risk of strangulation than inguinal hernias

Abdominal herniation may also occur as a result of iatrogenic injury to the abdominal musculature and inadequate wound healing (e.g. incisional hernia, parastomal hernia). Longitudinal studies have demonstrated that such hernias have increased in frequency over the last three decades now accounting for at least 5% of all abdominal wall hernias.[6]

In the early 19th century, the cause of herniation was thought to be a "mechanical disparity between the visceral pressure and the resistance of the abdominal musculature which was considered to be diminished by deficiency, debility or aging".[7] A common set of parameters (e.g. obesity, cough, constipation, pregnancy, etc) were considered causative but some investigators now suggest that these factors reveal rather than cause herniation.[7] A significant discovery in 1964 was the increased incidence of herniation in rats with defective collagen formation.[8] Further animal work involved the use of an iatrogenic agent (Beta-amino-proprionitrile, BAPN) which inhibits collagen cross-linking making it less durable.[8] Conner and Peacock in 1973 showed that transection of the internal inguinal ring led to hernia formation in 20% of cases. With BAPN alone, 6-10% of animals developed a hernia. However, transection of the internal ring and addition of BAPN increased the herniation incidence to 90%.[9] It became apparent that chemical mechanisms, as well as mechanical pathways, are important etiological factors in hernia development.

Chemically-mediated mechanisms of hernia formation are likely to involve altered collagen metabolism and impaired wound healing. In normal wound healing, an inflammatory response is initiated and blood fills the defect allowing proteins to form a provisional scaffold which directs incoming cells and organises the remodelling of the scar tissue. Fibroblasts are subsequently recruited to the area where they initiate and promote angiogenesis, collagen synthesis, extracellular matrix formation and granulation tissue. The latter is then remodelled and combined with foreign body giant cells to become fibrous scar tissue.[10]

Collagen is the end-product of fibroblastic activity with Type I and Type III having been implicated in wound healing and hernia formation.[11] [12] Type I is predominant in mature,

stronger wounds while the soluble, non-polymeric Type III dominates the early-stages of wound healing. The ratio of Type I/Type III defines the strength of the collagen strand, the fibril diameter and bundle architecture. A decreased ratio (less Type I or more Type III) is associated with herniation[13] and has been linked to increasing age, smoking and genetic disorders (e.g. Marfan's syndrome , Ehlers-Danlos).[7 11 14 15] The quality and amount of collagen in the body is also affected by the presence and amount of proteolytic enzymes (i.e. collagenases) and the lack of anti-enzymes that inhibit collegenase action. Smoking is a potent activator of collagenases.[7] Increased collagenase levels (e.g. metalloproteinase MMP2 and MMP9) have been reported in chronic human wounds, in compromised healing and in the elderly. Metalloproteinase inhibitors produce significantly stronger wounds even without any increase in collagen deposition. [16] Furthermore, reduced levels of metalloproteinase inhibitors (e.g. TIMP-1, TIMP-2) have been linked to the late occurrence of herniation[17-19]

Mechanical pathways involving a structurally and functionally impaired abdominal wall may also contribute to the progression of herniation. Skeletal muscle provides the bulk of the mechanical strength of the abdominal wall.[20] Skeletal muscle fibre development and regeneration are similar processes which involve mononuclear myoblasts that line up parallel to one another and fuse to form multinucleated myotubes. The myotubes undergo a maturation process with innervation and vascularisation to produce myofibers. Myofibers are then bound together by connective tissue to provide strength to the muscle and contract simultaneously when electrically stimulated resulting in voluntary movement.[21 22] When injury occurs, skeletal muscle regeneration and repair begins with the activation of progenitor cells, known as satellite cells, which migrate to the site of the defect and proliferate. Within the defect they align parallel to the injured myofiber and fuse to form

new myotubes which again undergo innervation and vascularisation to become functional myofibers.[10] Few studies have explored the association between hernia formation and muscle atrophy or degeneration.[23 24] Muscle atrophy leads to loss of contractile force and loss of muscle mass with reduced abdominal wall thickness.[24 25] In a rat animal model, hernia formation was associated with muscle atrophy, decreased cross sectional area and pathological fibrosis consistent with myopathic disuse atrophy. These changes occurred despite an increase in muscle collagen content suggesting a mechanical mechanism remote from chemically medicated pathways. [23] Interestingly, atrophic changes were more reversible following tension-free mesh hernioraphy than primary suture repair.[26]

1.3 Definition and incidence of parastomal herniation

A parastomal hernia is an incisional hernia related to an abdominal wall stoma.[27] Its precise incidence is unknown since published studies utilise a variety of clinical or radiological methodologies which have yet to be standardised (e.g. valsava manoeuvre, ultrasound, computed tomography) with additional uncertainty generated by variable follow-up intervals and heterogeneous cohorts[28]. A meta-analysis has estimated the incidence to be in the region of 30% for end-ileostomies and approximately 50% for end-colostomies after a 10-year follow-up period[27]. Studies have, however, reported the appearance of parastomal herniation 20 years post-operatively[5] and some surgeons believe it to be an inevitable consequence of stoma formation.[29] A colostomy appears twice as likely to herniate compared with an ileostomy. This could be related to the larger diameter of the trephine required to exteriorise the colon[27 30] although a recent study interestingly found different para-colostomy and para-

ileostomy herniation rates (46% versus 22% respectively) despite similar aperture sizes (median 30mm, range 20-50mm).[31]

1.4 Aetiology and risk factors for parastomal herniation

The development of parastomal herniation is associated with the presence of certain risk factors which may be classified as patient-related or surgery-related. Patient factors include increasing age, abdominal obesity, poor nutritional status, corticosteroid use, increased intra-abdominal pressure (due to chronic cough, constipation, benign prostatic hypertrophy, ascites), connective tissue disorders (e.g. Ehlers-Danlos syndrome) and other disorders that predispose patients to wound infection (e.g. diabetes mellitus).[31-35] A risk-stratification scoring system that takes into account the presence and influence of any these factors on the development of herniation might be a useful clinical tool to be developed since it may allow different management strategies for patients at low, medium, and high risk for herniation.

Surgical factors influencing the development of parastomal herniation include the diameter of the trephine, whether the stoma is constructed in an emergency setting and whether an intraperitoneal or extraperitoneal approach is used. [5 27 36 37]

Although insufficient evidence exists on the ideal trephine size for stoma formation, a defect of 3cm or more was found to be associated with a higher incidence of herniation.[31 38] Furthermore, for every millimetre increase in the aperture diameter, the potential herniation risk increases by 10%.[31] Traditional surgical teaching advocates creating a defect large enough to admit the tips of two fingers. This does not take into account the variability of surgeons' hand size. In fact, the average glove size of general surgeons is 7.5 [39] which

equates to the creation of an abdominal wall defect 3.5cm in diameter. Aperture size greater than 3.5cm has been found to be an independent predictor of hernia development on multivariate analysis,[31] thus the most common surgical technique frequently creates an oversized defect and does not allow the formation of a precise trephine according to the diameter of the exteriorised bowel segment.

Resnick first described the use of a mechanical device in an attempt to "control" the size of the abdominal trephine. The device consisted of three different size disposable heads (17, 25 and 32mm diameter) with a cartridge containing an annular knife and conical anvils.[40] The device allowed the creation of a precise abdominal defect with only one case of herniation out of 32 patients reported with a mean follow up of 7 years.[41] Other investigators have since used a circular stapler to construct colostomies with relative success.[42] [43]It is clear that although the optimum diameter of the trephine is unknown, an oversized defect, frequently created by the current surgical technique, is not only undesirable but may contribute in itself to the development of herniation. Circular stapling devices of various diameters may be advantageous in controlling the size of the abdominal wall defect. Their potential therapeutic value has not been assessed by randomised controlled trials.

Stoma formation via a trans-peritoneal or extra-peritoneal approach warrants further discussion. Goligher first described the extra-peritoneal stoma in 1958 and reported a herniation rate of only 9% with a follow-up of at least 2 years.[44] [45] Other studies reported that extra-peritoneal colostomy provided some protection against para-colostomy herniation but only one study demonstrated a statistically significant difference.[5] [46] A recent meta-analysis of 1,071 patients comparing the extra-peritoneal versus the intra-peritoneal route for permanent colostomies found a lower parastomal herniation rate in the extra-peritoneal arm

(odds ratio=0.41, 95% confidence interval=0.23-0.73, p=0.002)[47] There is, currently, insufficient level I evidence to advocate routine use of the extra-peritoneal technique since it is technically more difficult and time consuming, especially in a laparoscopic scenario, and requires further colonic mobilisation to provide extra length for the extra-peritoneal course. Furthermore, there are still concerns regarding the functional outcome of the stoma and the possibility of obstruction as the intestine follows its extra-peritoneal course.

The trans-peritoneal approach has been the most popular method of stoma formation over the last two decades.[48] This approach allows stoma formation either directly through or lateral to the rectus muscle. Sjodahl *et al* investigated the incidence of parastomal herniation in patients with permanent intestinal stomas formed either directly through or lateral to the rectus muscle. One hundred and seven patients had a stoma formed through the rectus abdominis with 23 patients lateral to it. The incidence of parastomal herniation was 2.8% and 21.6 % respectively.[49] Other studies have not confirmed these findings.[5 36 50] Furthermore, Stephenson and colleagues reported that the lateral rectus abdominis positioned stoma (LRAPS) was associated with only 10% parastomal herniation rate in 41 patients with a mean follow up of 23 months (range 19-29).[51] Despite the lack of sufficient evidence, stomas are routinely fashioned through the rectus muscle since this technique is not associated with any disadvantages.[1] Splitting and excessive stretching of the rectus fibres, however, is likely to damage and weaken the muscle and may be an important factor in the pathogenesis of herniation. Moreover, injury to the epigastric nerves, which supply the rectus abdominis muscles, as a possible mechanism has not been previously considered. Partial or complete nerve transection may lead to denervation of the rectus abdominis with resulting muscle atrophy and abdominal wall weakness.[52 53]

Emergency surgery has always been thought to increase the likelihood of parastomal herniation as the intraoperative trephine diameter often needs to be larger to safely exteriorise an obstructed dilated bowel segment and also because the finer technical aspects of stoma formation are not always the priority in life threatening emergency situations. Interestingly, a retrospective study evaluating transverse colostomies in 251 patients did not find any difference in parastomal herniation rates between emergency and elective surgery.[54] Other studies reported herniation rates of 2-4% for stomas formed in an emergency situation.[55 56] These figures are inconsistent with those most widely quoted in the literature and difficult to explain. They could, however, be related to patients lost to follow-up because of the higher mortality associated with emergency surgery or the fact that a certain number of stomas are reversed within months from the primary operation. For example, Mealy *et al* reported parastomal hernia incidence of 2.7% in 73 patients who underwent emergency stoma formation but the follow up time was not mentioned and the stoma closure rate was around 60%. [56]

In conclusion, the most popular surgical technique of stoma formation has several technical limitations including inability to create a consistent trephine diameter with potential damage to the rectus muscle and its associated blood/ nerve supply. All these may contribute to the high rates of parastomal herniation.

1.5 Diagnosis of parastomal herniation

Parastomal hernias can be diagnosed by clinical examination with the subject either supine with legs elevated or standing being asked to cough or strain.[57] The aim of the examination

is to demonstrate a positive cough impulse or palpable defect adjacent to the stoma. In patients with small parastomal hernias and/or abdominal obesity, clinical assessment may be difficult and equivocal. Computed tomography (CT) may be used, if clinically indicated, to increase the diagnostic accuracy. This also allows pre-operative classification to be made.[36] CT assessment with the patient in the prone position has been suggested in one study to improve the clinical and radiological reproducibility and correlation.[58] This is due to the hernia becoming more obvious due to gravity.

1.6 Classification of parastomal herniation

Devlin was the first person to classify parastomal hernias into 4 clinical subtypes[59]:

(i) subcutaneous, the hernia sac lays in the subcutaneous tissues,

(ii) interstitial, the hernia sac is within the abdominal wall layers,

(iii) peristomal, a bowel segment prolapses through a circumferential sac surrounding the stoma and

(iv) intra-stomal, as in case of ileostomies, the hernia sac is positioned between the intestinal wall and the everted intestinal layer.

The complexity of this system has limited use in surgical practice together with the abscence of data correlating the above subtypes with symptoms and surgical outcomes has made this classification impractical to use. A new radiological classification has been proposed by Moreno-Matias *et al* involving three subtypes according to the contents of the hernia sac: (i) type I, the hernia sac contains the stoma loop, (ii) type II, the hernia sac contains omentum and (iii) type III, the sac contains a bowel loop other than stoma.[60] Similarly, the usefulness

of such a classification system in surgical practice is debatable. Consequently, clinicians prefer to simply classify parastomal hernias as symptomatic and asymptomatic which has implications for the management of such patients.

1.7 Symptoms and Quality of life (QoL) with parastomal herniation.

Several studies have reported that stoma formation has a negative impact on quality of life (QoL).[61-67] It is thought that parastomal herniation has an even greater detrimental effect because it causes further change in body image and cosmesis, increased pain, difficulty with stoma appliance application resulting in leakage of bowel contents, obstruction and incarceration. Only two studies have assessed the effect of parastomal herniation on QoL. Kald *et al* used disease-specific and stoma-specific questionnaires to report a statistically significant reduction in QoL of patients with a parastomal hernia compared to patients without herniation.[68] Another study assessed, using regression modelling, predictors of poor quality of life in patients with diverting loop ileostomy after restorative proctocolectomy. It showed that parastomal herniation was a significant predictor of impaired QoL.[69]

Additional evidence supporting the hypothesis that parastomal herniation is associated with impaired QOL scores is provided by studies assessing patients with incisional hernias who did not undergo stoma formation. In a non-randomised study by Thaler *et al*, patients with incisional hernia after open or laparoscopic colectomy had significantly worse SF-36 scores for the domains of physical functioning, general and mental health and social functioning compared to patients without herniation.[70] Cheatham *et al* reported that patients with massive incisional hernias following abdominal decompression for intra-abdominal

hypertension demonstrated significantly decreased perceptions of physical, social and emotional health in comparison to the general population.[71]

Nevertheless, there is a paucity of high quality evidence assessing the impact of parastomal herniation on QoL. This highlights that surgeons have underestimated the effect of such a hernia on patients and may explain why little progress has been made in the reduction of this complication over the last few decades.

1.8 Treatment of symptomatic parastomal hernias.

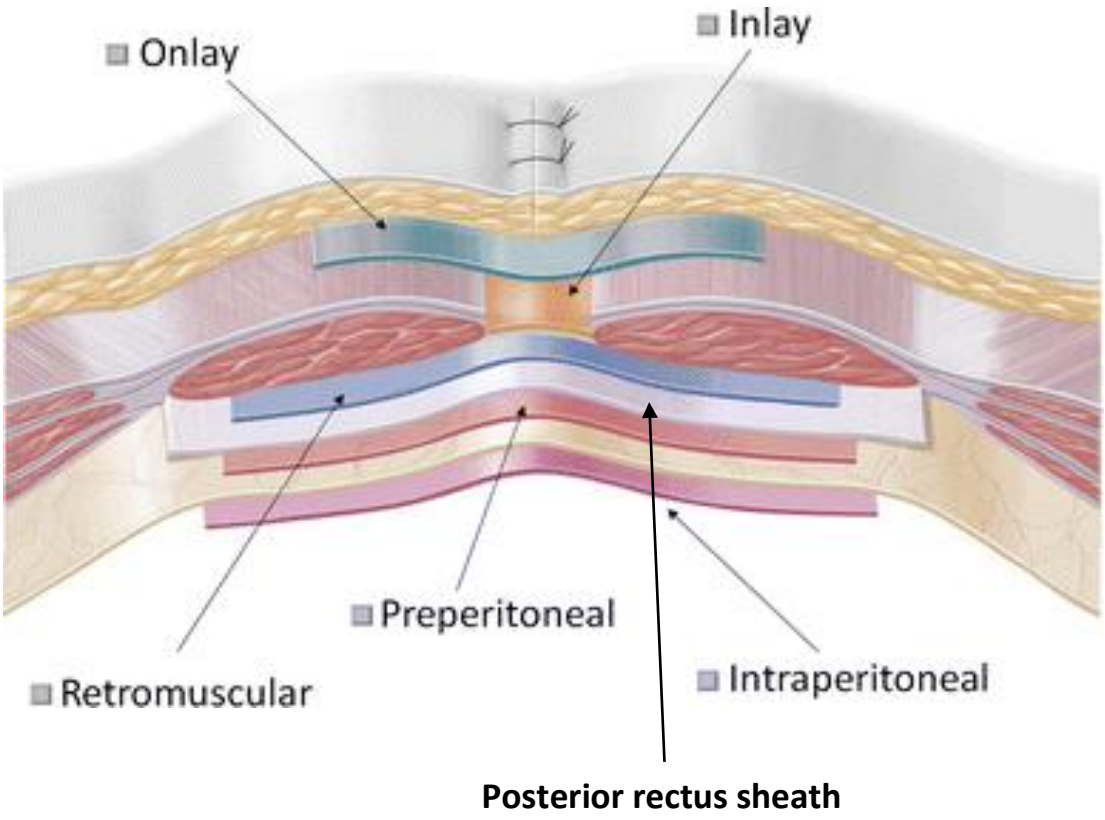
1.8.1 Open parastomal hernia repair

Patients with parastomal hernias require surgical repair in 11-70% of cases with most studies reporting rates of approximately 30%.[37 72] Pain and limitation of daily activities are considered by surgeons the most important indications for incisional/parastomal hernia repair whereas cosmetic complaints are viewed as less significant.[73] Nonetheless, current surgical techniques such as primary fascial repair or complete resiting of stoma are associated with very high recurrence rates and have the potential for significant morbidity and mortality.[27 37] Local aponeurotic repair recurrence rates range between 45% and 75%.[74-76] Stoma repositioning carries the morbidity and mortality associated with a major laparotomy and the recurrence rates are equally disappointing at approximately 50%.[1,3,24,26] Furthermore, resiting of the stoma may result in an additional incisional hernia at the site of the original stoma or the midline wound.[77 78] These procedures should not be routinely performed.

Following the success of mesh repair for other types of hernia, the technique of mesh reinforcement has naturally led to its use for the treatment of parastomal hernias.[79-101] Several techniques have been described involving implantation of the mesh in an on-lay, sublay, pre-peritoneal or intra-peritoneal plane in relation to the abdominal wall layers (Figure 1.1). The fascial onlay technique requires suturing the mesh to the anterior rectus sheath; the sublay (retromuscular) approach involves positioning the mesh between the rectus muscle and the posterior rectus sheath; the pre-peritoneal approach requires separation of the peritoneum and posterior rectus sheath and placement of the mesh in between and, finally, intra-peritoneal mesh placement involves attachment to the visceral peritoneal surface.

The fascial onlay technique was first described by Rosin and Bonardi more than 30 years ago[90] and since then studies with relatively poor methods of assessment (Level IV) have reported variable results with this technique (Table 1.1, page 32). In general, recurrence rates vary between 0 and 62.5% with the largest studies reporting rates of between 8% and 26%. In addition, infection rates are between 0 and 12.5% in the largest series with the mesh removal rates between 0 and 23%. In one of largest series with the longest mean follow up, the recurrent herniation rate for stomas reinforced with a polypropylene ring prosthesis was 16% but more importantly the mesh removal rate was the highest reported at 23%.[81]Overall, the onlay mesh reinforcement technique appears to be safe with acceptable infection rates and lower recurrence rates compared with fascial repair or stoma resiting. Further long- term studies are, however, required to assess the mesh explantation rate. The attraction of this technique is that a formal redo laparotomy may be avoided and should be considered in high risk patients with small/medium size hernias.

Figure 1.1 Diagrammatic illustration of mesh placement in relation to abdominal wall layers.



There are very few studies investigating mesh placement in the pre-peritoneal plane (between peritoneum and posterior rectus sheath) and sub-lay position (between rectus muscle and posterior sheath).[91-93] The theoretical advantages of placing the mesh in this anatomical plane are that the peritoneum prevents contact with bowel and the rectus muscle and the anterior rectus sheath prevent mesh "lift-off". Egun *et al* used pre-peritoneal mesh implantation without any recurrences in a series of 10 patients with a mean follow up of 54 months (range 22-69) but reported two superficial wound infections with one case of mesh explantation and subsequent stomal infarction.[92] Longman and Thompson reported no recurrences or complications with a sub-lay technique in 10 patients with a mean follow up of 30 months.[91] Kasperk *et al* also used a sub-lay technique but they reported 2 early recurrences among 7 patients which they attributed to technical errors (i.e. making mesh defect too large and using absorbable sutures that allowed mesh disruption).[93] Overall, it is very difficult to draw any meaningful conclusions from such a small series of studies with relatively short follow up but there were 2 recurrences in 27 patients (7.4%) and 1/28 (3.6%) cases of mesh removal (Table 1.2, page 34).

The intra-peritoneal approach was first described in 1980 by Sugarbaker who covered the fascial defect with a piece of synthetic mesh which was secured around its margin except laterally where the colon exited the abdominal cavity to form the ostomy.[100] The excellent results produced by Sugarbaker have not been replicated by all other investigators (Table 1.3). In a small series of 7 patients with a median follow up of 81 months, the recurrence rate was 28.6% and polypropylene mesh related complications included dense adhesions in 4 patients (57%) necessitating a laparotomy for intestinal obstruction in one patient.[97] In addition, one patient developed an intra-abdominal abscess 3 years post-operatively and the

mesh had to be removed with great difficulty because of the adhesions.[97] The risk of polypropylene mesh for significant adhesions, bowel erosion and fistulation has led other investigators to use PTFE (polytetrafluoroethylene) mesh. PTFE is a synthetic mesh which is softer, more pliable with smaller pores and appears less likely to cause bowel erosion and adhesions.[94 102 103] The recurrence rate was between 0% and 15% but the studies all had a short follow-up (Table 1.3, page 35). Importantly, complications such as adhesions, bowel erosion and fistulation were not reported. However, PTFE has pores measuring less than 10μm with the potential for infection as they could harbour bacteria.[94] Longer term follow up studies are required to assess its infective complications.

Table 1.1 Outcomes following on lay mesh repair for parastomal hernia. Studies listed in chronological order.

Study	Year	Level of Evidence	No Repairs	% Recurrence	% Infection	% Erosion	% Mesh Removal	Follow up (mean)	Type of assessment
Luning[79]	2009	IV	16	19.0	6.2	0.0	6.2	6-110 (33)	not stated
Guzman-Valdivia[80]	2008	IV	25	8.0	8.0	0.0	0.0	8-24 (12)	clinical
De Ruiter[81]	2005	IV	46	15.9	6.6	-	22.7	12-156 (60)	clinical
Kanellos[82]	2004	IV	4	0.0	0.0	0.0	0.0	(36)	not stated
Steele [83]	2003	IV	58	26.0	3.4	2.0	0.0	0.2-139 (50.6)	clinically
Geisler[84]	2003	IV	16	62.5	12.5	6.2	6.2	2-161 (39)	Phone survey+ case notes
Venditti[101]	2001	IV	8	0.0	12.5	0.0	0.0	(36)	not stated
Amin [85]	2001	IV	9	0.0	0.0	0.0	0.0	3-12 (6)	not stated
Kald[86]	2001	IV	5	20.0	0.0	0.0	0.0	(12)	not stated

Tekkis[87]	1999	IV	5	0.0	0.0	0.0	0.0	9-38 (21.4)	Not stated
Bayer [88]	1986	IV	7	0.0	28.5	0.0	14.3	(48)	not stated
Abdu [89]	1982	IV	5	0.0	20.0	0.0	0.0	24-48	not stated
Rosin [90]	1977	IV	7	0.0	0.0	0.0	0.0	3-48	not stated

Table 1.2. Outcomes following sub-peritoneal mesh repair for parastomal hernia.

Study	Year	Level of Evidence	No Repairs	Mesh position	% Recurrence	% Infection	% Erosion	% Mesh Removal	Follow up (mean)	Type of follow up assessment
Longman[91]	2005	IV	10	sublay	0.0	0.0	0.0	0.0	2-40 (30)	Case notes+ patient contact
Egun[92]	2002	IV	10	Preperitoneal	0.0	20.0	0.0	10.0*	22-69 (54)	not stated
Kasperk[93]	2000	IV	7	Sublay	28.6§	0.0	0.0	0.0	4-36	not stated

* For stomal infarction post ARDS, §due to technical error

Table 1.3. Outcomes following intraperitoneal mesh placement.

Study	Year	Level of Evidence	No Repairs	Mesh	% Recurrence	% Infection	% Erosion	% Adhesions	% Mesh Removal	Follow up (mean)	Type of follow up assessment
Ballas[94]	2006	IV	2	PTFE	0.0	0.0	0.0	0.0	0.0	24-60 (42)	CT
Van Sprundel[95]	2005	IV	15¶	PTFE	13.3	0.0	0.0	0.0	0.0	5-52 (29)§	clinical
Stelzner[96]	2004	IV	20	PTFE*	15.0	5%	0.0	5.0	0.0	3-84 (42)	Clinical ± US ± case notes
Morris -Stiff[97]	1998	IV	7	Polypropylene	28.6	14.3	0.0	57.0	14.3	60-89 (81)§	not stated
Hofstetter[98]	1998	IV	13	PTFE*	0.0	0.0	0.0	0.0	0.0	Over 96	not stated
Byers JM[99]	1992	IV	9	Polypropylene	0.0	11.1	0.0	0.0	0.0	(13.4)	Retrospective case note review
Sugarbaker[100]	1980	IV	7	Polypropylene	0.0	0.0	0.0	0.0	0.0	48-84	not stated

*Polytetrafluoroethylene mesh, ¶ one laparoscopic excluded § median,

1.8.2 Laparoscopic parastomal hernia repair.

With the rising popularity of laparoscopic surgery over the last two decades, its use has spread to the treatment of parastomal hernias.[104-117] The rationale is that the laparoscopic approach is associated with minimal additional injury to the abdominal wall and potentially offers a superior view of the defect allowing more precise repair and reinforcement with a mesh.[106] [107] Several investigators have reported their experience with laparoscopic parastomal hernia repair with variable success rates (Table 1.4). The conversion rates vary between 0 and 15%. Most laparoscopic repair studies report low wound infection rates of 0-5% with low mesh explantation rates (up to 10%). The most popular mesh in the laparoscopic studies was the Polytetrafluoroethylene (PTFE) which may account for the very low rate of erosion between 0 and 1.5% (*Table 4*). Although PTFE is a soft, inert material with minimum reactivity that does not adhere to bowel,[103] its major drawback appears to be its tendency to shrink,[106] [107] which accounts for the disappointing recurrence rates of up to 46% (Table 1.4, page 39). The shrinking of PTFE mesh is thought to be due to the small pore size of the mesh which prevents tissue in-growth and incorporation (see section 1.10.2).[102] Hansson and colleagues reported that in almost all patients in their series who were re-operated for a recurrent parastomal hernia, the mesh appeared smaller with a wider central opening which was likely to be the cause for the recurrence.[107] This is clearly an important observation since their series is one of the largest with a median follow up of 36 months. The ability of the PTFE mesh to provide effective, long term treatment for parastomal hernias is in doubt and warrants further investigation. Other investigators have used instead of the "keyhole" technique, variations of the "Sugarbaker technique" that avoids creating a central hole with a slit in the mesh. Mancini *et al* reported recurrence rates

of 4% with this technique[112] whereas Pastor *et al* [108] reported recurrence rates of 29% using the modified 'Sugarbaker' technique and 67% using a 'keyhole' technique. Similarly, Berger and Bientzle reported 8 recurrences in 41 patients (19.5%) using the modified 'Sugarbaker' technique which they thought was disappointing. Subsequently, they used a two-mesh sandwich technique in the next 25 patients with no reported recurrences but the median follow up was only 12 months.[110]

An increasing number of symptomatic parastomal hernias may be repaired laparoscopically. However, the ability of PTFE, which has been the mesh of choice in the published literature, is in doubt because it shrinks by almost 50% and has the potential to cause long-term septic complications.[118] One way of possibly reducing the risk of infectious complications is by using antimicrobial impregnated meshes.[119] The problem of mesh shrinkage can possibly be addressed by the use of composite meshes commonly made of polypropylene and PTFE although other materials such as polyvinylidene fluoride (PVDF), cellulose and omega-3 fatty acids have been used. The PTFE interface allows safe intra-peritoneal placement with minimal adhesion formation and the additional surface ensures strong adherence to the abdominal wall by inducing a fibrotic reaction.[118] However, the long term results with such meshes are not known. There is also evidence that adhesions are prevented in the short term but the effect diminishes with time.[120] In addition, the two layers can become separated allowing bowel adherence.[121]

In conclusion, the laparoscopic approach appears attractive in view of the theoretical advantages of a more precise repair, minimal injury to the abdominal wall and faster postoperative recovery with decreased postoperative pain. Nevertheless, patients occasionally may complain of pain from the tacking sutures or clips to the abdominal wall.

Laparoscopic parastomal hernia repair is associated with similar results to open repair and, hence, the benefit of this approach is unclear in terms of the longevity of repair considering the reported problems with mesh shrinkage and surgical repair technique. If a laparoscopic approach is selected then "Sugarbaker/modified Sugarbaker" or "Sandwich" techniques should be the preferred therapeutic options, since, at the present time, they appear to be superior compared to the "keyhole" technique.

Table 1.4. Recurrence and complication rates following laparoscopic mesh placement

Study	Year	Level of Evidence	No Repairs	% Conversion	Type of Mesh	Technique	% Recurrence	% Infection	% Erosion	% Mesh Removal	Follow up (mean)	Type of follow up assessment
Mizrahi H[104]	2011	IV	29	6.9%	PP§/PTFE	*Keyhole*	46.4	3.4	0.0	3.4	12-53 (30)*	Clinical ± CT if available
Wara[105]	2010	IV	66	4.0	PTFE/PP	*Keyhole*	3	4.5	1.5	6.0	6-132 (36)*	Clinical + CT if in doubt
Hansson [106] [107]	2009	IV	54	14.5	PTFE	*Keyhole*	37	1.8	0.0	3.7	12-72 (36)*	Clinical ± CT/US
Pastor [108]	2009	IV	12	8.3	PTFE	*Keyhole/Sugarbaker*	33.3	16.6	0.0	0.0	(13.9)	not stated
Muysoms[109]	2008	IV	24	0.0	Polyester/PTFE /PP	*Keyhole/Sugarbaker*	41.7	0.0	0.0	0.0	4-54 (21.2)	clinically ± CT
Zacharakis[117]	2008	IV	4	0.0	PTFE	*Keyhole*	25%	0.0	0.0	0.0	9*	clinical
Berger [110]	2007	IV	66	1.5	PVDF¶/PP	*Sugarbaker/"Sandwich"*	12	4.5	0.0	3.0	3-72 (24)*	not stated
Craft [111]	2007	IV	21	0.0	PTFE	*Keyhole/Sugarbaker*	4.7	4.8	0.0	9.5	3-36 (14)	Case notes review
Mancini [112]	2007	IV	25	0.0	PTFE	*Sugarbaker*	4.0	4.0	0.0	4.0	2-38 (19)*	Clinical
Le Blanc [113]	2005	IV	12	0.0	PTFE	*Keyhole/ Sugarbaker*	8.3	0.0	0.0	0.0	3-39 (20)	not stated
Safadi[114]	2004	IV	9	0.0	PTFE	*Keyhole/slit*	44.4	0.0	0.0	0.0	6-33	clinical
Kozlowski [115]	2001	IV	4	0.0	PTFE	*Modified Sugarbaker*	0.0	0.0	0.0	0.0	2-33	clinical

Voitk[116]	2000	IV	4	0.0	Polyprolene	*Sugarbaker*	0.0	0.0	0.0	0.0	2-12	not stated

*median §Polypropylene ¶polyvinylidene fluoride

1.8.3 Emergency parastomal hernia repair

There is a paucity of data in the literature regarding emergency parastomal hernia repair. No studies have been identified that have specifically evaluated outcomes following emergency treatment for incarcerated or strangulated parastomal hernias. Such an operation has the potential for significant morbidity and mortality[122] and frequently requires a laparotomy, bowel resection and stoma resiting which, as mentioned previously, is associated with high recurrence rates. The majority of surgeons would commonly avoid using a synthetic mesh in the presence of intestinal ischemia and bowel resection but studies evaluating emergency paraumbilical, incisional and inguinal hernia repair with mesh have reported good outcomes with low post-operative complications.[123-125] [126] The safety of synthetic meshes for emergency parastomal hernia repair requires further evaluation but this is a situation where biological meshes may potentially be advantageous in view of their ability to be used in infected or contaminated fields.[127] This may be off-set by their significant economic cost at present.

1.9 Prevention of parastomal herniation

The high incidence of parastomal herniation together with the unsatisfactory results of its repair and morbidity associated with any corrective operation has led to a novel approach with emphasis on prevention. Consequently, some investigators have instituted the use of a "prophylactic" mesh at the time of the initial operation to prevent the development of herniation.[128-130] Three randomised controlled trials have shown that implantation of a prophylactic mesh in the pre-peritoneal or sublay position is associated with a reduction in

parastomal herniation when compared to standard unreinforced stoma formation.[72 131 132] Two studies used a synthetic mesh (*Vypro*® or Ultrapro®) to reinforce the stoma trephine in patients with permanent end colostomies.[72 132] In the third study, a porcine collagen implant (Permacol®) was used to reinforce the trephine in patients with defunctioning loop stomas.[131] (Table 1.5)

A recent meta-analysis showed that the herniation rate in patients with synthetic mesh reinforced end-colostomies (8 of 55, 14.5%) was lower when compared to the standard group (32/54, 59.2%, RR 0.24, 95%CI 0.05 to 1.22; p=0.08).[133] Similarly, the percentage herniation in Permacol® reinforced loop ileostomies (0/10, 0.0%) was lower than the conventional group (3/10, 30%, RR 0.14, 95% CI 0.01 to 2.45; p=0.18). More importantly, there was a reduction in the percentage of clinically detected parastomal hernias requiring surgical treatment. Thirteen percent of patients with conventional end colostomy underwent repair of parastomal hernia compared to none in the reinforced group (RR 0.13, 95%CI 0.02 to 1.02; p=0.05). There was no difference in stoma related morbidity or mortality.[133] Evaluation of morbidity is particularly important because there is concern among surgeons regarding the use of synthetic mesh near bowel as there is a perceived risk of septic complications from bacterial contamination of the mesh, adhesions, intestinal obstruction and fistulation. Two studies did not report any mesh related infections or mesh explantation.[72 131] One study reported three midline laparotomy wound infections in the mesh group (3/27, 11.1%) but the overall infection rate was identical to the conventional group (3/27, 11.1%) and no mesh had not be removed. Stoma related morbidity (e.g. peristomal infection, necrosis) was also similar in both groups.[132] Thus, the evidence demonstrates that mesh rejection does not appear to be a major issue. This may be partly

attributed to the new generation of biologic or synthetic meshes which are better incorporated by the tissues and are more resistant to bacterial infection.[4] Placement of the mesh in the pre-peritoneal or sublay position protects the mesh from bacterial contamination and minimises contact with the bowel, thus further decreasing the risk of infection, adhesions or fistulation.

Prophylactic reinforcement of the abdominal wall trephine with a mesh appears to be a promising possible solution to parastomal herniation. The results of the previously reported randomised controlled trials should be interpreted in the context of important limitations. Serra-Arracil *et al* reported a 26% reduction in the clinical incidence of herniation in the treatment group but patients were excluded from the study if they had a BMI>35, cirrhosis, COPD and corticosteroid treatment.[132] In fact, it is these high risk factors which are associated with parastomal/incisional herniation. In addition, the study by Hammond *et al* had small numbers with a median follow up of only 6.5 months.[131] Finally, in Janes' study the herniation rate in the control arm was high (81%), the drop-out rate was 6/27(22.2%) in the control group but double in the mesh group (12/26, 46.15%) and the radiological incidence of herniation was not reported.[72] Synthetic meshes (e.g. Vypro®, Ultrapro®) have been shown to reduce the incidence of parastomal herniation without added morbidity and are relatively inexpensive.[72 132] There is limited evidence in the literature regarding the prophylactic use of biological meshes which are considerably more expensive but are thought to be less prone to infection due to their biocompatibility. The infection rates, however, following prophylactic reinforcement are low and, at the moment, the higher cost and lack of evidence regarding their relative efficacy over synthetic ones prohibits their routine use for prophylactic stoma reinforcement.

Table 1.5. Summary of RCTs assessing the effect of prophylactic mesh reinforcement on the prevention of parastomal herniation.

Study	Year	Level of Evidence	No of patients in each arm (standard versus mesh)	Stoma Type	Mesh type	Mesh position	(%) Clinical Recurrence (standard versus mesh)	Stoma related morbidity
Hammond[131]	2008	II	10 vs 10	Loop ileostomy	Permacol®	Between peritoneum and rectus sheath	30.0 vs 0.0	None reported
Janes[72]	2009	I	27 vs 27	End colostomy	Vypro®	sublay	81.0 vs 13.3	None reported
Serra-Aracil[132]	2009	I	27 vs 28	End colostomy	Ultrapro®	sublay	40.7 vs 14.8	7.4% both arms

1.10. The ideal surgical mesh

The ideal mesh for the treatment or prevention of parastomal herniation is currently unknown. In theory, the chosen material should be sterile, non carcinogenic with adequate strength to resist increased intra-abdominal pressures while it stimulates tissue remodelling and regeneration. It should also be "biocompatible" with the surrounding tissues by producing a favourable interaction between host and implant without causing acute/chronic inflammation or seroma formation. From a surgical prospective it should also have the following properties:

- Easy to handle
- Antibacterial and resistant to chronic infection
- Promote tissue re-growth and re-modelling while preventing bowel adhesion and fistulation
- Inert with minimal contraction
- Available at a reasonable cost for routine use

1.10.1. Types of surgical meshes

Over the last 30 years numerous materials have been described for hernia repair with more than 70 meshes now available on the market for abdominal wall reconstrustion.[134 135] Such mesh implants can be classified as synthetic (absorbable or non-absorbable) or biological.

1.10.2 Synthetic meshes

1.10.2.1 Non-absorbable (permanent) meshes

Permanent meshes can be classified according to composition, filament structure (monofilament or multifilament) and pore size (microporous <75μm or macroporous>75μm). They are all manufactured from three basic surgical materials: monofilament polypropylene, multifilament polyester and expanded polytetrafluoroethylene (ePTFE) in combination with each other or with a range of other materials such as titanium, omega-3, and hyaluronate. Monofilament polypropylene was first introduced in the 1960s and still remains the most commonly used material for surgical meshes because of its hydrophobic nature and resistance to bacterial colonisation.[136] Monofilament meshes offer the advantages of high tensile strength with low infection rates but they are rigid with decreased abdominal wall conformity.[137] Multifilament meshes are less rigid but more prone to infection.[138] Macroporous meshes allow greater tissue in-growth and biocompatibility but are more likely to produce adhesions. Microporous implants are less adhesiogenic due to the fact that they become encapsulated but they are more prone to infection as the small pores cannot be assessed by macrophages. [118]

The original thinking behind the use of a mesh was that the material should be very strong to reinforce the abdominal wall while inducing a fibrotic reaction and scar tissue formation.[118] Meshes such as Marlex®, Surgipro®, Prolene® (monofilament polypropylene) and Dacron®, Mersiline® (multifilament polyester) are heavy-weight, macroporous and they produce an intense fibrotic reaction with the potential for pain, movement restriction and bowel complications (e.g. adhesions, fistulation).[118 139-141] It became apparent that the surface

area, and hence strength of the mesh, had to be reduced. Calculations of the intra-abdominal pressure revealed that this was possible without compromising mesh function since the required tensile strength to withstand maximum abdominal pressure is only a tenth of that of most meshes.[142 143] This led to the concept of light-weight, partially absorbable meshes such as Vypro® (polypropylene interwoven with absorbable Vicryl) and Ultrapro® (polypropylene interwoven with Monocryl). The light-weight meshes stimulate a reduced inflammatory reaction with greater biocompatibility. They also shrink less (Vypro shrinks 29% and Ultrapro <5%) and are associated with less pain, bowel adhesion, erosion and fistulation.[118] Despite these improvements, many surgeons remain wary of such implants in close proximity to the bowel.

The search for the "ideal" mesh led to the development of alternatives such as expanded polytetrafluoroethylene (ePTFE) and composite meshes. ePTFE (GoreTex®, MycoMesh® and DualMesh®) is inert, hydrophobic and its implantation produces less severe inflammatory reaction and tissue ingrowth than polypropylene.[144 145] Furthermore, its microporous nature means that it becomes encapsulated, thus allowing intraperitoneal placement. However, it shrinks by 40-50% with time accounting potentially for weaker hernia repair (please see section 1.8.2, Laparoscopic parastomal hernia repair) and is associated with higher incidence of infective complications.[118] Its encapsulation and small pore size (<10μm) prohibit the host immune system from reaching harboured microbes, thus necessitating mesh removal when infection occurs.[118 144 146 147] Its use in contaminated or potentially contaminated fields is not recommended. Manufacturers have attempted to improve the properties of PTFE mesh by producing variations with full-thickness pores (MycoMesh®), with a textured parietal surface to improve tissue incorporation

(DualMesh®) and with an antibacterial coating (DualMesh® Plus). There is no evidence that these products alleviate the concerns associated with the use of PTFE.[148]

Composite meshes consist of a visceral and a parietal surface with the former permitting safe intraperitoneal placement with reduced adhesiogenesis and the latter promoting tissue in-growth and intergration.[118] The parietal surface usually consists of polypropylene and the visceral surface of PTFE (Composix®), PVDF (polyvinylidene fluoride,; Dynamesh®) or a cellulose-based material (Proceed®, Sepramesh®). The long-term results with composite implants are unknown with some studies showing that, despite their use, bowel complications such as adhesions still occur. This may be related to the surgical technique used for mesh fixation, a diminished anti-adhesional effect with time or separation of the two layers at the edges and exposure of the polypropylene layer to the bowel.[149]

1.10.2.2 Absorbable meshes

Absorbable meshes contain glycolic acid and (poly)lactic acid compounds at different ratios and their degradation increases with increased glycolic acid content.[150] The most commonly used absorbable mesh is made of polyglactin (Vicryl), a combination of glycolic and lactic acids in a ratio of 9:1. This mesh loses 50% of its strength within 2-3 weeks whereas an implant made predominantly of lactic acid (95% lactic acid and 5% glycolic acid) maintains its strength for at least 9 months.[151] [152]Absorbable implants are thought to provide mechanical support in the acute phase with subsequent fibro-connective tissue formation taking over the repair in the long-term.[144] In view of this property, they have excellent

biocompatibility and low risk of infective or bowel-related complications.[118] [153] Their theoretical advantages are not accompanied by satisfactory results with long-term data indicating that that they are not any better than simple suture repair.[144] [154] Furthermore, in an animal model, Tyrell *et al* did not observe any hernia recurrences with non-absorbable mesh but all ventral hernias recurred within 10 weeks of repair with absorbable implant.[155] There is currently no evidence to support their use in abdominal wall reconstruction.

1.10.3 Biological meshes

The problems encountered with synthetic implants led to the use of biomaterials which were first introduced in the 1990s for soft tissue reconstruction.[156] These products provide the extracellular scaffold required for tissue reconstruction by promoting angiogenesis and proliferation of fibroblasts and myocytes resulting in deposition of new extracellular matrix.[156] Biological meshes can be classified as allografts (derived from human tissue) and xenografts (usually derived from porcine or bovine tissue).[157] Tissues commonly used include dermis, intestinal submucosa and pericardium which undergo complete de-cellularisation to produce a three dimensional collagen structure which is biocompatible and acts as scaffold for host cell population, vascularisation and tissue remodelling.[157] [158] However, premature collagenase degradation, especially in infected or contaminated fields due to increase enzymatic activity, may lead to implant resorption before adequate tissue re-growth has taken place. This may be the reason that the herniation rates associated with these materials are comparable to that of absorbable synthetic grafts.[155] [159-161] Supplemental

chemical cross-linking (with gluteraldehyde, hexamethylene diisocyanate[HDMI], etc) has been used since 1975 to improve biological mesh stability and resistance to enzymatic breakdown with the theoretical disadvantage of decreased host-tissue integration.[160 162 163] The different cross-linking agents produce different cross-linking structures with variation in the mechanical strength and performance of the collagen matrix.[156] Table 1.6 summarises some of the most commonly used and available biological meshes available for parastomal hernia repair. [131 164-168]

The number of studies in the literature investigating the use of biological implants for the treatment of parastomal herniation has increased over the last few years but the quality remains modest, at best, with almost all studies being level IV.[158] In a systematic review of the literature designed to assess the use of collagen-based implants for the repair of parastomal hernias, Slater *et al* showed that the overall recurrence rate was 16% after a median follow up of approximately 1 year. In addition, the rate of wound-related complications was 26.2% with no graft infections or explantations.[158] These results are comparable to those achieved using synthetic meshes. It has been suggested that the great attraction of "biological scaffolds" is that they can be used in infected or potentially-infected surgical fields with explantation unnecessary for the resolution of infection.[169 170] The infection and explantation rates though associated with the use of synthetic meshes for the treatment or prevention of parastomal herniation are low.[133] Consequently, the high cost of the biological implants (Table 1.6) coupled with the potential to lose their mechanical strength (e.g. Surgisis, non cross linked products) and the inability to demonstrate any advantages over synthetic meshes cast significant doubts on their routine use for the treatment or prevention of parastomal herniation.

Table 1.6 Most commonly available biological meshes

Mesh	Type	Manufacturer	Cross-linked	Cost/ cm^2 ($)
AlloDerm	Human dermis	LifeCell	No	35.31
Strattice	Porcine dermis	LifeCell	No	26.00
Permacol	Porcine dermis	Covidien	Yes	18.97
Surgisis	Porcine intestine	Cook	No	20.00
Veritas	Bovine Pericardium	Synovis	No	22.02
Tutopatch	Bovine Pericardium	Tutogen	No	-
Periguard	Bovine Pericardium	Synovis	Yes	3.91

1.11 Conclusion

Parastomal hernation is an important but unappreciated health care issue with significant negative impact on patients' lives. Its treatment can be very challenging so avoidance of this complication is desirable. Prevention of herniation by prophylactic mesh reinforcement is a novel approach and, despite more studies required to draw defitive conclusions, it appears to be safe and should be offered to all patients undergoing routine stoma formation, especially if they are at high risk. Further research, however, is required to address the aetiopathogenesis of parastomal hernia formation as until we have fully understood the mechanism of its formation, direct prevention and treatment will always be unsatisfactory. Future studies for the prevention of parastomal herniation will need to explore the use of biological/synthetic mesh using a standardised technique that is easy to use in both open and laparoscopic surgery. The study should have in the follow-up assessment radiological evaluation (e.g. CT ± valsava manoeuvre) of the stoma site to allow measurement of the true incidence of herniation and objective evaluation of any preventive interventions. The duration of follow up should be at least five years as it has been demonstrated that 80% of parastomal hernias appear within this time frame.[72]

1.12 Aims and objectives of this thesis

The main aims of this thesis are:

1. To establish the true incidence of parastomal herniation in patients with permanent stomas and its impact on quality of life (QoL) and health care resources.

2. To correlate parastomal herniation with trephine size as this might facilitate the development of appropriate size stapling devices that control the trephine size and reduce the incidence of herniation.
3. To establish the safety, reproducibility and efficacy of a novel surgical technique in reducing the incidence of parastomal herniation compared to standard stoma formation.
4. To explore, using biochemical and radiological means, the contribution of the rectus abdominis muscle to the development of parastomal herniation by assessing its ability to repair after surgery.

CHAPTER 2

Correlation of the radiological incidence of parastomal herniation with the diameter of the abdominal wall defect.

2.1 Introduction

Parastomal herniation is the most common complication of permanent stoma formation. The precise incidence is unclear but rates between 5% and 80% have been reported in the literature reflecting the different forms of assessment utilised at varying follow-up intervals.[27 72] Radiological evaluation of the stoma site with computed tomography (CT) has been used as an aid to improve the diagnostic accuracy.[171] In a small study (n=23), CT detected the rate of parastomal herniation to be 78% whereas the clinical herniation rate was only 52%.[78] In another prospective series of 27 patients the CT-detected rate of parastomal herniation was marginally higher compared to the clinical rate (44.4% versus 40.7% respectively) after a median follow up of 29 months.[132] Thus, CT evaluation of the stoma site appears to be superior to clinical examination alone for the detection of parastomal herniation.

The multifactorial aetiology of parastomal herniation is well documented (section 1.3) but only increasing age and abdominal wall defect size have been found to be independent predictors of its development on multivariate analysis.[31] There is, however, a lack of data regarding the ideal trephine size with only one clinical study reporting higher rates of para-colostomy herniation with an abdominal wall defect diameter greater than 35mm.[31] Due to the lack of evidence, the majority of colorectal surgeons still continue to create a manual trephine large enough to accommodate the exteriorised bowel segment. The average glove size of general surgeons is 7.5 which equates to the creation of an abdominal wall defect at least 3.5cm in diameter.[39] Consequently, the most common surgical technique is not "custom-fit" to the bowel size and is associated with the risk of creating an oversized defect, which may contribute in itself to the development of herniation.

The precise dimensions of the trephine were first considered to be an important factor by Resnick who used a circular stapling device of various diameters (17, 25 and 32mm) to create a trephine with only one case of herniation in 32 patients after 7 years.[40 41] Other investigators have used standard circular stapling devices to construct colostomies without any increase in stoma related morbidity.[42 43]

The aim of this study was to assess the radiological incidence of parastomal herniation in patients who had a permanent end-colostomy for malignancy and to correlate it with the size of the abdominal wall defect. This may allow identification of a stoma defect size that minimises the risk of para-colostomy herniation, provide further evidence about the importance of avoiding an oversized defect and contribute to the design of appropriate size stapling devices that facilitate trephine formation.

2.2 Methods

All patients who underwent permanent end-colostomy formation as part of a Hartmann's procedure or abdomino-perineal excision of the rectum (APER) for malignancy between January 2004 and December 2009 at a large specialist tertiary colorectal unit (Barts' and the London NHS trust) were identified from a departmental cancer registry. Patients' demographics (age, gender, body mass index [BMI]), operative details (date of surgery, type of surgery, emergency/elective setting) and any stoma related symptoms were recorded. Post-operative abdominal computerised tomography (CT) scans performed for clinical purposes were reviewed by a single consultant radiologist for evidence of parastomal herniation. A parastomal hernia was defined as an incisional hernia related to the stoma

site.[33] Furthermore, a parastomal hernia was classified as symptomatic if patients experienced faecal leakage due to poor adherence of stoma bag, pain, discomfort or developed complications such as bowel obstruction or incarceration secondary to the para-colostomy hernia.

For patients without any radiological evidence of herniation, the latest CT scan was used to measure the maximum diameter of the abdominal wall defect in any direction. Patients with confirmed para-colostomy hernia on radiological assessment had the maximum aperture diameter measured using the earliest scan, in chronological order, in which a hernia could be identified.

The data were analysed using a commercially available statistical analysis software (GraphPad Version 5, GraphPad Software Inc, La Jolla, CA). Data normality was tested using the De Agostino–Pearson omnibus normality test. Intergroup comparison of variables was performed using a Mann-Whitney U test. A p value <0.05 was considered significant.

2.3 Results

A total of 59 patients underwent an open or laparoscopic Hartmann's procedure or APER for malignancy over a 5-year period. All colostomies were fashioned using a trans-peritoneal approach. Sixteen patients did not have any post-operative CT scans available for review and were excluded from the final analysis. The study group consisted of 43 patients (22M: 21F) with a mean age of 69 years. (Figure 2.1)

There were 25 patients (58%) with radiological evidence of parastomal herniation after a median follow up of 26 (range 6-55) months. Eighteen patients did not have any evidence of para-colostomy herniation on CT assessment after 16 (range 7-49) months. The difference in the follow up interval of the two groups was not statistically significant (p=0.11). The two groups were also of similar age and although patients with parastomal hernias had a higher BMI (26.9 versus 23.5), it was not statistically different (p=0.24) (Table 2.1). Furthermore, the number of stomas formed following an emergency Hartmann's procedure was comparable between the two groups. (Table 2. 1)

The median maximum diameter of the abdominal defect for patients with a parastomal hernia was 35mm (range 25-58mm). This was found to be statistically larger (p<0.0001) than the median diameter of the group without herniation (22mm, range 7-36mm). Among patients with radiologically confirmed parastomal hernias, 11/25(44%) were symptomatic. The characteristics of this sub-group are shown in Table 2.2. Four patients (36%) with symptomatic parastomal hernias underwent surgical repair. The maximum trephine diameter in patients with a symptomatic parastomal hernia was 54mm (range 28-58mm) and was not statistically different (p=0.06) when compared to the trephine size of patients with an asymptomatic parastomal hernia (34mm, range 25-55mm).

Figure 2.1. Study flow chart

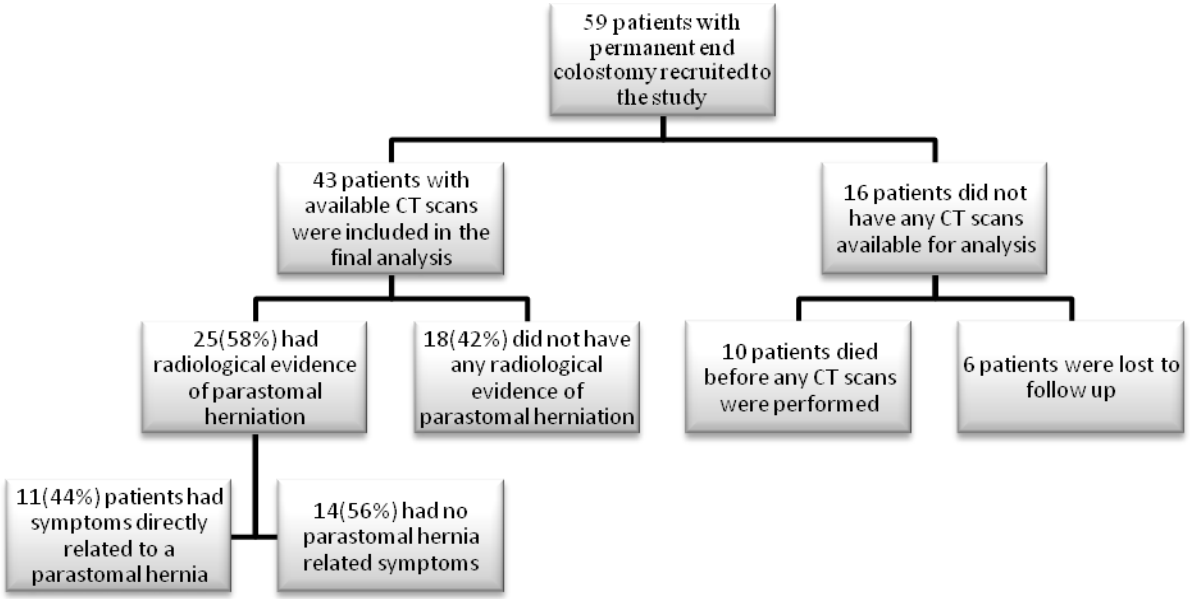


Table 2.1. Intergroup comparison of patient variables based on the presence of parastomal herniation

Demographics	Parastomal Hernia	No parastomal hernia	p-value
Total Number	25	18	-
Mean Age	69±14	69±12	0.89
Female: Male Ratio	14:11	7:11	-
BMI	26.9 (20.0-36.0)	23.5 (22.0-30.0)	0.24
Hartmann's procedure	13 elective 4 emergency	10 elective 3 emergency	-
APER	7	5	
Median defect diameter(mm)	35 (25-58)	22 (7-36)	<0.0001
Median time of post-op CT(months)	26 (6-55)	16 (7-49)	0.11

Table 2.2. Comparison of abdominal wall defect size between symptomatic and asymptomatic parastomal hernia.

Demographics	Symptomatic parastomal hernias	Asymptomatic parastomal hernias	p-value
Total Number	11	14	-
Mean Age	68±12	70±12	0.76
Female: Male Ratio	6:5	9:5	-
BMI	23 (21.5-36)	26.5 (20.0-35.0)	0.90
Median defect Diameter (mm)	54 (28-58)	34 (25-55)	0.06
Corrective surgery	4 (36%)	0	-

2.4 Discussion and conclusions

The incidence of parastomal herniation in this study was 58% , one of the highest reported in the literature.[27] Two possible reasons for this include firstly, the study group consisted of relatively older patients (mean age 69 years) with a diagnosis of malignancy, both of which are well known risk factors for herniation, and secondly, the presence of parastomal herniation was based on CT evaluation which is the most sensitive means of assessment. Forty-four percent of patients with a radiologically confirmed parastomal hernia had symptoms directly related to it and 36% of them required surgical repair whereas the majority opted to be managed conservatively or were deemed to be high risk for surgical intervention. Emergency surgery does not appear in this study to be associated with an increased risk for parastomal hernia development. This is in agreement with another study evaluating transverse colostomies in 251 patients, where no difference in para-colostomy herniation rates was found between emergency and elective surgery.[54] However, the number of patients is small so any firm conclusions will require further evaluation.

Although patients with a parastomal hernia had statistically a larger abdominal wall defect when compared to patients without herniation, a distinct "cut –off" point between the two groups was not identified. Nevertheless, no cases of para-colostomy herniation were seen with an abdominal wall defect measuring below 25mm. This should be taken into consideration when a trephine is fashioned since a previous study reported that for every millimetre increase in aperture size, the risk of developing a hernia increases by 10%.[31] The most common technique of stoma formation, using the crude method of finger measurement, does not allow the creation of a precise trephine diameter. The use of a

circular stapling device might be advantageous in forming a more controlled, rigid trephine which maintains its size and integrity with time. [40 172]

A limitation of this study though is its retrospective nature and relatively small study sample. However, most publications in this area involved small populations. More importantly, measurement of the size of the abdominal wall defect in the presence of herniation is associated with the ambiguity of whether the hernia caused the oversized defect or vice versa. We attempted to reduce the influence of this factor by using the first scan in which a parastomal hernia was identified. In either case, neither the abdominal wall defect size in hernia-free patients (median 22mm), nor the fact that no herniation was observed with diameter below 25mm should be affected by this factor.

In conclusion, the majority of patients who undergo end-colostomy formation for colorectal malignancy appear to develop a para-colostomy hernia within the first two post-operative years. Aperture size has been previously shown to be a potential independent predictor of herniation[31] but our study suggests that creating a defect ≤25mm might reduce this risk.

CHAPTER 3

A case-controlled pilot study assessing the safety and efficacy of the Stapled Mesh StomA Reinforcement Technique (SMART) in reducing the incidence of parastomal herniation.

"The high incidence of parastomal herniation is unacceptable. All colorectal surgeons have a duty to prevent it".

Professor Norman Williams, 2010

President, Royal College of Surgeons of England

3.1 Introduction

Various technical modifications (e.g. extra-peritoneal stoma formation, stoma positioned lateral to the rectus abdominis muscle) have been proposed as a means of reducing the incidence of herniation but there is no conclusive evidence that such manoeuvres are effective. Moreover, traditional stoma formation, using manual dilatation to create the abdominal wall defect, frequently results in an oversized aperture when a trephine ≤25mm is associated with reduced herniation risk.[173]

Three previous randomised controlled trials showed that prophylactic mesh reinforcement of the trephine can reduce the incidence of parastomal herniation. In two studies, a synthetic mesh (Vypro® or Ultrapro®) was placed as sublay (behind the rectus muscle) at open surgery. In the third study, a porcine collagen implant (Permacol™) was implanted preperitoneally (between peritoneum and posterior rectus sheath) because of the fear of erosion. The latter was expanded into a multi-centre trial in the United Kingdom with the acronym "Propheci" (**Pro**phylactic **P**arastomal **He**rnia **C**linical **I**nvestigation, ISRCTN31730807). Unfortunately recruitment to this trial was slow leading to its suspension. Manual mesh implantation is perceived to be time consuming and unnecessarily cumbersome, particularly at the end of a long and challenging operation. In addition, an increasing number of stomas are being constructed laparoscopically and sub-peritoneal placement of the mesh has proved difficult by this approach. Consequently, routine stoma reinforcement is not standard practice in the United Kingdom and in most countries worldwide.

A simple and quick technique is therefore required for stoma formation which addresses the issues of aperture size and reinforcement. The technique should also be easily reproducible

at both open and laparoscopic surgery. Utilisation of a circular stapling device to create a controlled trephine and simultaneously reinforce it with mesh may deal with all technical issues and simplify the reinforcement process.

The aim of this study was to assess the safety and efficacy of a novel surgical technique called SMART (**S**tapled **M**esh stom**A** **R**einforcement **T**echnique) in reducing the incidence of parastomal herniation.

3.2 Materials and Methods

3.2.1 The "SMART" technique for the prevention of parastomal herniation.

The **S**tapled **M**esh stom**A** **R**einforcement **T**echnique (**SMART**) utilises a circular stapling instrument of various knife diameters (17mm, 20mm and 24mm) to create the trephine and simultaneously reinforce it with mesh.

3.2.1.1 "SMART" at open surgery

Trephine formation commences by excising a cylinder of abdominal wall skin and subcutaneous tissue down to the rectus sheath. The sheath is then opened with a cruciate incision (Figure 3.1) and the rectus muscle is gently split in the line of its fibres (Figure 3.2). The anvil of an appropriate sized, purpose designed circular stapling gun (Compact^{TM}, Chex Healthcare) is then introduced via the open abdomen. The diameter of the gun depends on the diameter of the bowel which will eventually traverse the stoma trephine. A purpose designed grasper is then inserted via the abdominal wall trephine to penetrate the posterior rectus sheath and peritoneum. The grasper is used to grasp the anvil

Figure 3.1 The rectus sheath is opened by means of a cruciate incision.

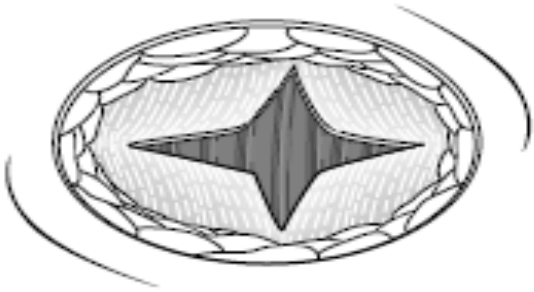
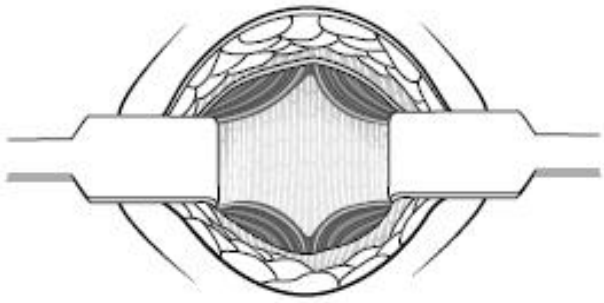


Figure 3.2 The rectus fibres are gently retracted to reveal the posterior rectus sheath



shaft of the gun and is designed not to damage it. The grasper tip is blunted but sufficiently sharp to penetrate the layers it needs to transgress. Consequently, visualisation of its tip when penetrating the abdominal wall is vital. Once the anvil shaft has been grasped the anvil is then exteriorised through the trephine to emerge on the abdominal wall (Figure 3.3). Another purpose designed grasper is used externally to grasp the anvil shaft and steady it and facilitate its eventual mating with the spike emanating from the cartridge housing component of the instrument. The collagen mesh (Permacol™) which is configured in a circular design with a diameter of 7cm is then prepared by creating a small defect in its centre. The defect in the mesh is utilised to insert the mesh onto the anvil shaft which is then mated with the stapler housing spike of the Compact™ instrument (Figure 3.4). Once successful locking has been achieved the gun is closed, whilst the rectus muscle fibres are gently retracted, enclosing in order the mesh, the posterior rectus sheath and the peritoneum. The gun is then fired and removed leaving behind a precise, rigid trephine with the mesh stapled in the posterior rectus sheath. The circumference of the mesh is next sutured to the anterior rectus sheath with interrupted 0 PDS sutures so it lies flat against the anterior sheath and totally lines the trephine for 2-3cm circumferentially through the split muscle fibres (Figure 3.5). The colon or ileum is then drawn through the trephine and the stoma is fashioned in the usual way (Figure 3.6).

Figure 3.3 The anvil of an appropriate sized COMPACT™ stapler is introduced via the open abdomen. The anvil shaft is then grasped with a purpose designed instrument configured to prevent damage to the shaft and is then exteriorized through the trephine to emerge on the abdominal wall.

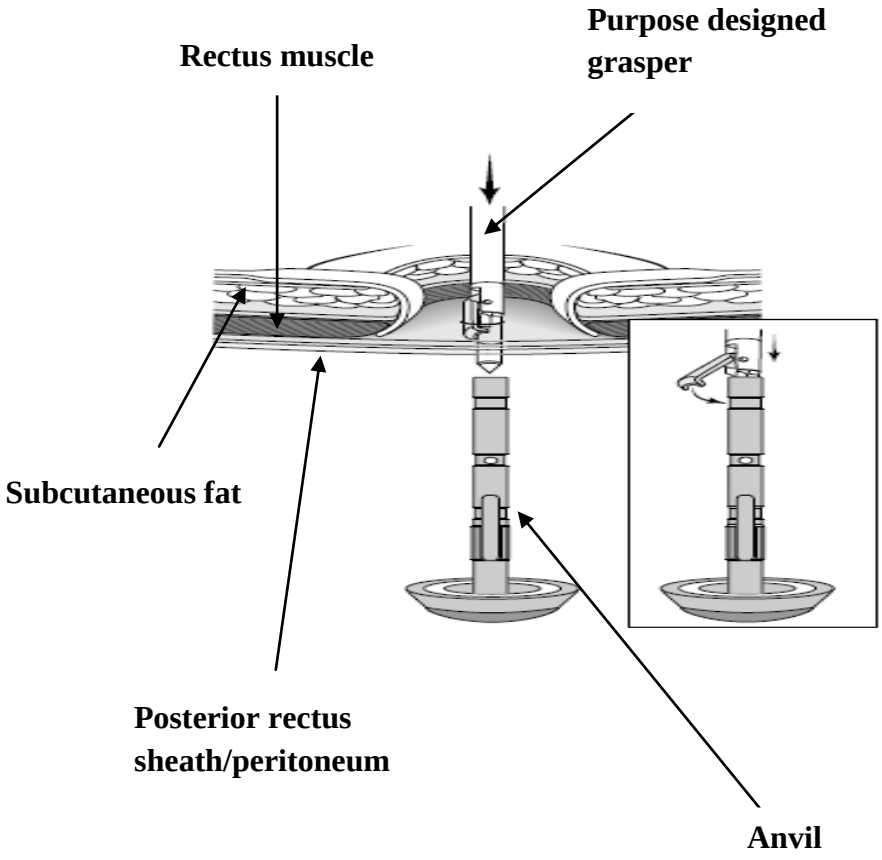


Figure 3.4 The anvil shaft, with a previously prepared circular mesh of 7 cm in diameter, is stabilized with a purpose designed right angled grasper. It is then mated with the trocar of the stapling gun. The gun is closed, fired and withdrawn leaving a reinforced trephine (see Fig 3.5).

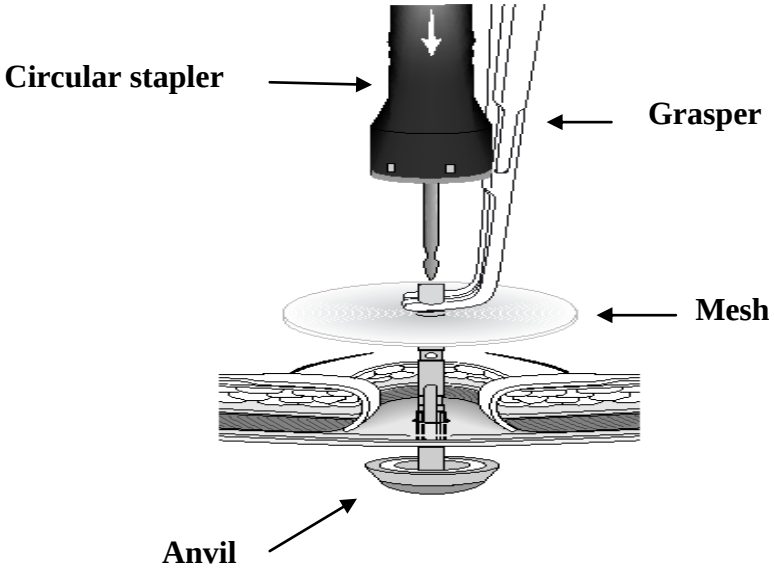
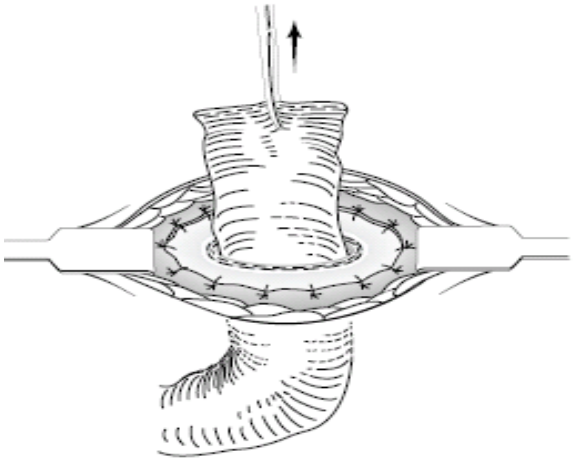


Figure 3.5 The circumference of the mesh is next sutured to the anterior rectus sheath.

Figure 3.6 The colon or ileum is finally drawn through the trephine and the stoma is fashioned.



3.2.1.2 "SMART" at laparoscopic surgery

With the pneumoperitoneum still maintained the abdominal wall trephine is formed down to the posterior rectus sheath as described above for the open technique. If a separate abdominal wall incision has been created for specimen retrieval the anvil shaft can be inserted into the abdominal cavity and exteriorised in the same way as for the open technique. If this is not the case the rectus muscle fibres can be retracted and a small incision made via the abdominal wall trephine in the posterior rectus sheath and peritoneum. This naturally results in deflation of the abdominal cavity as the pneumoperitoneum is lost. A prolene or PDS purse-string suture is next inserted into the edge of the incision in the peritoneum and posterior rectus sheath. The anvil head is passed through this incision with the shaft exteriorised and the purse-sting is tied firmly around its base on the shaft. Mating between the anvil shaft carrying the mesh and the stapler housing spike is then completed as described previously and the gun is closed. Before firing, the pneumoperitoneum is re-created and the trephine site and position of the closed gun is checked to ensure no extraneous segment of bowel has been trapped between the anvil and the posterior abdominal wall. After firing the gun is withdrawn and the stoma constructed in the usual way (please see supplementary DVD demonstrating the SMART procedure)

3.2.2 Study Design

All patients who underwent the SMART procedure in our institution from 2011 onwards were identified from a prospectively recorded computerised database. Patients were offered the procedure on clinical grounds as being at particularly high risk for parastomal herniation with randomisation into a controlled trial being deemed inappropriate. All

patients gave informed consent and understood that this was a new variation of an established technique. The study was reviewed and approved by the National Research and Ethics Committee (West London REC Reference Number 10/H0706/92).

Data recorded included patient demographics (age, gender), body-mass index, American Society of Anaesthesiologists (ASA) grade, indication for SMART and postoperative complications including parastomal hernia formation. The diagnosis of herniation was made clinically in the outpatient department by an independent reviewer blinded to the procedure. In patients with stoma related symptoms (e.g. peristomal pain) computed tomography (CT) was used to confirm the presence or absence of herniation.

Patients who declined SMART and opted to undergo stoma resiting to the opposite side of the abdomen without mesh reinforcement for symptomatic parastomal herniation during the same time period were used as a control group. All patients in both groups received prophylactic antibiotics at induction of anaesthesia. Bowel preparation and surgical drains around the stoma site were not routinely used.

Statistical analyses comparing the SMART and control groups for the measurable parameters were performed using a commercially available software package (GraphPad Version 5, GraphPad Software Inc, La Jolla, CA). Data normality was assessed using the De Agostino-Pearson omnibus normality test. Normally distributed data are presented using mean and standard deviation, whereas non-normal data are presented as a median and range. Normally distributed data were compared using paired t-tests. Analysis of non-normal data was performed using the Mann-Whitney U tests**.** A p value of <0.05 was considered statistically significant.

3.2.3 Patients and Indications

Twenty-two patients (16F:6M, mean age 49±16, BMI 33.0±7.0) underwent stoma formation with SMART (18 open: 4 laparoscopic; 11 ileostomies:11 colostomies). All SMART stomas were fashioned using a circular stapler with a 24mm knife diameter. Patients presented with either complications from a pre-existing stoma (n=15) or underlying conditions (n=7) such as obesity, asthma, corticosteroid use, collagen disorder or combination of these. The group of patients with pre-existing stomas had either a large parastomal hernia unsuitable for local repair (n=6) or recurrent herniation as a result of previous repair (n=9) and all of them underwent resiting to the opposite site of the abdomen. Patients (n=7) with underlying conditions predisposing them to herniation underwent SMART at the index operation.

The four patients who underwent the laparoscopic technique were all females who required a stoma for severe faecal incontinence (n=3) or slow transit colon (n=1). In addition, 3 of them had associated recurrent full thickness rectal prolapse and 2 of them were also thought to have a collagen disorder and weak abdominal wall musculature.

The control group consisted of 11 patients (6F:5M, mean age 59±15, BMI 29.0±3.0) with statistically similar age and body-mass index to the SMART group (Table 3.1). All control group patients underwent stoma resiting with no reinforcement (4 ileostomies:7 colostomies) to the opposite site of the abdomen for symptomatic parastomal herniation.

Table 3.1 Demographics, physiological parameters and surgical outcome for the SMART and control groups.

Variables	SMART group n=22	Control Group n=11	p-value
Age	49±16	59±15	0.1
Gender ratio (F:M)	16:6	6:5	-
ASA grade			
I	2	0	
II	8	3	-
III	12	8	
IV	0	0	
BMI (Kg/m^2)	33.0±7.0	29.0±3.0	0.1
Stoma Type			
Ileostomy (%)	11(50)	4(36)	0.48
Colostomy (%)	11(50)	7(64)	0.48
Risk factor			
Parastomal Hernia	n=15	n=11	-
Other (e.g obesity)	n=7	n=0	
Approach			
Laparoscopic	4	0	-
Open	18	11	
Recurrence Rate	4 (18%)	8(73%)	**0.003**
Follow-up (months)	18 (10-24)	9 (4-25)	**0.04**

3.3 Results

There were no intra-operative complications or immediate stoma related post-operative complications in either group. In the SMART group, there was one death on the 12th post-operative day from respiratory sepsis in a patient with significant co-morbidities including advanced multiple sclerosis. Two further patients, both in the SMART group, were re-admitted within 30 days: one had radiological drainage of a pelvic collection and the second underwent band adhesiolysis (40 cm proximal to the stoma) for small bowel obstruction. Both patients recovered uneventfully.

The majority of patients in the control group (8/11, 73%) developed a parastomal hernia within the first postoperative year (9 months; range, 4-25). At a median follow–up of 21 months (range 12-24), 3/22 (14%) SMART patients reported parastomal symptoms. CT evaluation confirmed recurrent herniation in all of them, one of which required reoperation. It should be noted that the recurrences occurred in a patient that required cardiopulmonary resuscitation for myocardial infarction following hospital discharge, in a patient that required radiological drainage of pelvic collection twice and in a patient with a diagnosis of Ehlers-Danlos syndrome and significant colonic dysmotility. A further patient without any stoma related symptoms was diagnosed with herniation on clinical examination, resulting in an overall recurrence rate of 18% (4/22) significantly lower than that in the control group (p=0.003) but with longer follow-up (Table 3.1). The other 18 SMART patients were asymptomatic without any clinical evidence of parastomal herniation or any other stoma complication during the follow up period.

3.4 Discussion and conclusions

This pilot study has demonstrated that SMART is safe and reproducible and appears to reduce the incidence of parastomal herniation in a high risk group of patients. It should be emphasised that although the two groups were comparable in terms of age, body-mass index and stoma type, SMART patients were thought to be at significantly higher risk for herniation than those in the control group because some of them had a recurrence of a previously repaired parastomal hernia and/or a combination of conditions (e.g. collagen disorder, asthma) predisposing to herniation. Furthermore, they were followed up for almost two years whereas all control patients who developed a parastomal hernia did so during the first postoperative year. The high herniation rate seen in the control group is similar to that reported by other studies providing further evidence that stoma resiting without reinforcement should not be performed for such patients as recurrence is almost inevitable.[174]

Stoma trephine formation using a circular stapling instrument was first described by Resnick at open surgery.[40] The present technique differs in several important aspects. It combines the concepts of stapling trephine creation with mesh reinforcement which has been shown to significantly reduce parastomal hernia rates. It allows construction of the stoma trephine to be created at open and laparoscopic surgery and simplifies the reinforcement process. A circular stapling instrument which is shorter than conventional instruments and more easily manoeuvrable with a longer trocar shaft facilitates the procedure, particularly in obese patients with a thick abdominal wall.

The reasons for the apparent efficacy of SMART to prevent parastomal herniation in this study warrant further discussion. The conventional technique for stoma formation involves stretching of the defect through the abdominal wall to accommodate the breadths of the surgeons' index and second fingers.[29,174] Such uncontrolled stretching of the abdominal wall not only may produce an oversized defect but may also result in excessive stretching of the rectus muscle which is likely to weaken the trephine with subsequent widening of the defect over time and retraction of the anterior rectus sheath leading to hernia formation. SMART may minimise the herniation risk by (i) controlling the size of the defect (all stomas fashioned with a circular stapler with 24mm knife diameter) (ii) reinforcing the abdominal wall with mesh and (iii) minimising excessive stretching of the rectus muscle.

It should be emphasised that SMART was used only in patients who were at high risk for herniation and for whom randomisation was deemed inappropriate in view of the findings from previous mesh reinforcement trials. [72, 131, 132] The predisposition of patients in this study towards herniation is, perhaps, the main reason accounting for the 18% recurrence rate in just under 2 years although this is significantly lower than the herniation rate (73%) in the control group.

Limitations of this prospective study include its non-randomisation, the heterogeneity of the SMART cohort and the lack of longer term follow-up (>5 years). Nevertheless, it provides important data that SMART is safe and reproducible and has the potential to replace the current surgical technique for stoma formation since it addresses many technical deficiencies associated with the latter. A change in surgical practice, however, will require definitive evidence from an adequately powered multicentre randomised controlled trials

assessing the efficacy and cost-effectiveness of SMART in patients undergoing routine stoma construction during open or laparoscopic surgery (please see Chapter 4).

CHAPTER 4

A randomised controlled trial of Stapled Mesh stoma Reinforcement Technique ('SMART') versus standard technique to assess effect on parastomal herniation.

4.1 Introduction

The high incidence of parastomal herniation and the generally unsatisfactory outcomes associated with its open or laparoscopic repair (Chapter 1, Section 1.8) necessitate a different approach with the emphasis on prevention. As discussed in Chapter 1 (Section 1.9), Level 1 evidence suggests that insertion of a prophylactic mesh at the primary operation is safe and reduces the herniation rate.[72 131 132] There is a need, however, for further large randomised controlled trials in all patients undergoing routine permanent stoma formation. Previous studies were either underpowered or inappropriately excluded patients at higher risk for herniation (e.g. overweight, steroid users). Furthermore, standardising and simplifying the technique of stoma formation and reinforcement is another important factor that needs to be addressed in order to improve acceptability and adoption of the mesh reinforcement procedure which is still not routine practice in the United Kingdom or abroad, partly due to the difficulty associated with laparoscopic mesh implantation and the additional operative time required to do so.

A pilot study of the **S**tapled **M**esh stom**A** **R**einforcement **T**echnique ('SMART') has demonstrated it be safe and reproducible in a group of patients who underwent stoma resiting, predominantly for recurrent symptomatic parastomal herniation (Chapter 3). Nevertheless, SMART needs to be tested in a multicentre randomised controlled trial in all patients who undergo permanent stoma formation for benign or malignant disease. In the pilot study, SMART was performed using Permacol® mesh (i.e. a cross-linked collagen implant) because of the fear of bowel erosion with synthetic meshes and the perceived merits of biological materials which provide a collagen scaffold for tissue repair and regeneration in potentially contaminated surgical fields (Chapter 1, section 1.10.3). In view

of the lack of evidence regarding the superiority of biological meshes over synthetic ones and the significantly higher cost of the former, it can be argued that implantation of a synthetic mesh might be of similar efficacy while preventing the cost of the procedure spiralling to unacceptably high levels, especially in the current climate of limited healthcare resources worldwide. [157 158]

The primary aim of this study was to assess the efficacy of SMART in reducing the clinical herniation rate compared to the standard technique following permanent stoma formation. Secondary objectives included: (i) to compare the radiological incidence of herniation between the two techniques and correlate it with the clinical findings, (ii) to measure differences in complications associated with the two techniques, (iii) to assess the ease of the SMART technique compared with the standard technique and, (iv) to compare the quality of life between patients who underwent SMART and standard stoma formation.

4.2 Materials and Methods

The study was approved by the National Research and Ethics Committee in the United Kingdom (NREC, West London REC 3, 10/H0706/92) and by local Research and Development departments at participating sites. The sponsor was Queen Mary University of London.

4.2.1 Inclusion and Exclusion criteria

All patients requiring a permanent colostomy or ileostomy for benign or malignant bowel disease, as part of an elective open or laparoscopic procedure, were prospectively invited to participate in the study. Patients were over 18 years old, gave fully informed written consent and agreed to the randomisation procedure. Females of childbearing potential were also required to provide a negative pregnancy test.

Patients were excluded from the study for one or more of the following reasons:

(i) Taking part in another clinical study directly relating to this one.

(ii) Having a history of parastomal herniation. Such patients were deemed inappropriate for randomisation and were offered alternative surgical options with reinforcement (e.g. SMART, stoma resiting).

(iii) Suffering from an untreated metabolic or systemic illness (e.g. diabetes or rheumatoid arthritis or any immunological disease).

(iv) Diagnosed with a mentally limiting condition such as Alzheimer's.

(v) Having MRSA or clostridium difficile infection.

(vi) Having abdominal wall sepsis

(vii) Pregnancy

4.2.2 Randomisation

All patients who satisfied the inclusion/exclusion criteria and gave informed consent were enrolled into the study. Randomisation was performed on the day of surgery, following induction of anaesthesia, by means of opening consecutively numbered sealed envelopes, to

receiving either a standard stoma without reinforcement or a 'SMART' stoma. Patients were blinded as to which arm of the trial they had been entered. Un-blinding was only performed in case of complications, if necessary.

4.2.3 Surgical technique

4.2.3.1 Established technique

The ileostomy or colostomy was formed using the surgeon's index and middle finger to create a defect in the posterior rectus sheath which was then stretched, as required, to allow safe exteriorisation of the bowel segment.

4.2.3.2 The Stapled Mesh stomA Reinforcement Technique ('SMART')

The 'SMART' procedure for open and laparoscopic surgery has been described in detail previously (Chapter 3, Section 3.2). All such procedures were performed using the same circular stapling device (COMPACT ™, CHEX HEALTHCARE). The mesh used was Vypro II® (ETHICON products worldwide, a Johnson and Johnson company) measuring 15x15 cm, made from approximately equal parts of absorbable polyglactin multifilament thread and non-absorbable polypropylene multifilament thread. After absorption of the polyglactin component only the polypropylene component of the mesh remains. The Vypro II® mesh was chosen because of its relatively reasonable cost, general availability but also safety and efficacy in reducing parastomal herniation as shown in a previous study with five year

follow-up.[72] The diameter of the mesh implanted was initially 7cm but is was subsequently increased to 12 cm to allow reinforcement of greater peristomal surface area.

4.2.4 Power and sample size calculation

Power is the probability of correctly rejecting a false null hypothesis. Using a 2-group test of equal proportions, sample size estimation for the study was based upon a 1-sided, 5% significance level (alpha) and an 80% power. According to the literature on the prevalence of parastomal hernias, the clinical herniation rate in the control group was assumed to be 40% at year 1 with the rate in the SMART group being 15%. For the statistical power to be 80%, 58 patients per each treatment arm were required. It was therefore intended to recruit 116 patients undergoing permanent stoma formation allowing for an approximate 15% loss due to dropouts (e.g. death, loss of follow up).

4.2.5 Data collection

Preoperative data collection included demographics (age, sex, body mass index), relevant medical and surgical history (e.g. respiratory/connective tissue disorder, use of steroids, diagnosis of diabetes mellitus, smoking status), pre-operative albumin, American Society of Anaesthesiologists (ASA) grade, and indication for surgery. Preoperative quality of life was assess using a validate questionnaire, EQ-5D (Appendix 1).

Intra-operative date collection included surgical approach (open or laparoscopic), type of stoma (standard or SMART, ileostomy or colostomy), diameter of circular stapler used and

time taken for stoma formation. The ease of the technique was assessed by the operating surgeon using a liner analogue scale (1=difficult to 5=easy).

Post-operative data collection included duration of post-operative stay, time taken for stoma to work, general complications (e.g. ileus, wound infection, chest infection), and stoma related complications (parastomal hernia, haemorrhage, prolapse, retraction, obstruction, stenosis). Post-operative quality of life was also assessed using the EQ-5D questionnaire.

4.2.6 Assessment of primary end point

Patients had their ostomies examined by clinicians blinded to the surgical procedure performed. The primary end point was the development of parastomal herniation on clinical examination at 12 months postoperatively. Computerised tomography scans were also performed at 12 months to identify possible subclinical herniation and to correlate the clinical and radiological findings and obtain objective evidence on the efficacy of the 'SMART' procedure.

4.2.7 Statistical analysis

All statistical analyses were performed using a commercially available software package (GraphPad Version 5, GraphPad Software Inc, La Jolla, CA). Data normality was assessed using the De Agostino-Pearson omnibus normality test. Normally distributed data have been presented using mean and standard deviation, whereas non-normal data have been presented as a median and range. Normally distributed data were compared using paired t-

tests. Analysis of non-normal data was performed using the Mann-Whitney U tests**.** A *p* value of <0.05 was considered statistically significant

4.3 Results

4.3.1 Patients Demographics

At the time of completing this thesis, 50 of the 116 patients required, according to the power calculation, were recruited to the study but only 40 of them had completed their 12 month postoperative assessment and were included in this analysis. The study flow chart is shown in Figure 4.1.

Twenty patients underwent SMART (n=16 open and n=4 laparoscopically) and 20 had standard stoma formation (n=16 open and n=4 laparoscopically). One patient (ASA grade 3 with diagnosis of malignancy) in the SMART group (5.0%) died within the first postoperative year because of disease progression. In the control arm, one patient (5.0%) who underwent an abdomino-perineal excision of the rectum for malignancy died within the first 30 postoperative days because of respiratory sepsis. They were both excluded from the final analysis. The demographic characteristics of the remaining patients that were followed up according to the study protocol are shown in Table 4.1. Patients in the two arms were of similar age (65±11 vs. 71±13 years, p=0.27), body mass index (27±6 vs. 26±9 kg/cm^2, p=0.62) and nutritional status (preoperative albumin 39±8 vs. 39±9 g/dl, p=1.0). Stomas were predominantly fashioned for malignancy (84.2 % in the control arm and 68.4% in the SMART arm).

4.3.2 General complications

In the control group, 2 patients (10.5%) had superficial infection of the laparotomy wound that was treated successfully with antibiotics. A further two patients (10.5%) had an intra-abdominal collection that required radiological drainage. Finally, another two patients (10.5%, one with intra-abdominal collection), developed a postoperative chest infection that was treated with antibiotics. Two patients in total (10.5%) developed postoperative ileus that resolved spontaneously.

In the 'SMART' group, two patients (10.5%) were diagnosed with post-operative ileus with resolved after 6 and 9 days respectively and another patient (5.3%) developed a post-operative chest infection that was treated with antibiotics. A further patient (5.3%) developed perineal wound infection and dehiscence following an abdomino-perineal excision of the rectum and was treated successfully with antibiotics and VAC therapy. A final patient (5.3%) had pelvic bleeding following panproctolectomy for ulcerative colitis that required a laparotomy twice without any impact on the stoma site.

4.3.3 Stoma-related morbidity.

There were no stoma related complications such as stenosis, prolapse, or retraction in the control arm. One patient (5.3%), however, developed peristomal infection with was treated successfully with a course of antibiotics.

In the SMART group, the stoma-related complication rate was also 5.3%. This was due to one patient diagnosed with necrosis of the distal 2-3cm of the exteriorised bowel segment in the first 24 post-operative hours and required resection of the necrotic segment without

the need for a laparotomy. He made an uneventful recovery and he did not have a parastomal hernia at 12 months. There were no other complications such as infection or fistulation in the 'SMART' arm and no mesh had to be removed.

4.3.4 Parastomal herniation rates

The clinical herniation rate in the control group at 12 months was 36.8% (7 of 19 patients) and all hernias were confirmed radiologically. There were no subclinical parastomal hernias detected on CT evaluation. None of the parastomal hernias in the control group were symptomatic or required surgery. Four patients (21.0 %) had a clinical recurrence in the 'SMART' group with an additional patient diagnosed with a subclinical parastomal hernia on CT yielding a radiological herniation rate of 26.3%. The reduction in the clinical (p=0.02) and radiological herniation rate (p<0.05) was statistically significant. It should be emphasised that all cases of clinical herniation in SMART group occurred with mesh diameter of 7cm. Following implantation of a mesh 12cm in diameter there was only one subclinical case of herniation among 6 patients (16.7%) which was diagnosed on CT. At the one year follow-up none of the recurrent parastomal hernias in the SMART group required re-operation.

4.3.5 Technique Evaluation

Surgeons graded standard stoma formation with 4 and above on the linear analogue scale in 18 cases (94.7%) with only one procedure (5.3%) given a grade 2. In the test arm, 'SMART' was graded with 4 and above in 42.1% of cases (n=8). It was thought to be of

average difficulty (grade 3) in 5 cases (26.3%), while it was found to be difficult to perform (grade 1 and 2) in 5 cases (26.3%).

The mean time taken to perform a stoma was statistically similar between the two treatment arms ('SMART'=22±8 min, 'Standard'=22±9 min, p=0.91).

4.3.6 Quality of Life measures

Patients who underwent standard stoma formation had a mean preoperative score 76±9 on the EQ-5D questionnaire which was statistically unchanged at the 12-month assessment (68±9, p>0.05). The mean preoperative score for 'SMART' patients was 53±20 and improved to 65±8 ($p<0.05$) after 12 months.

Mobility was unaffected in the control group with 60% reporting no problems pre- and and post operatively. In the SMART group, the percentage of patients reporting increased mobility increased from 40% to 55% (p>0.05) at the 12 month assessment point.

In the self-care domain of the questionnaire, 100% of patients in the control group reported no problems but at the follow-up point only 80% (p=0.04) reported no problems. In the SMART group, 70% of patients had no issues preoperatively with hygiene and this proportion remained appeared unchanged (75% p=0.08). A decrease in the percentage of patients reporting no problems with usual activities , pain and anxiety was seen in both the control and SMART arms of the study but the reduction was smaller in the SMART group, albeit not statistically significant (p=0.2).

Figure 4.1 Study flow chart of the SMART randomised controlled trial.

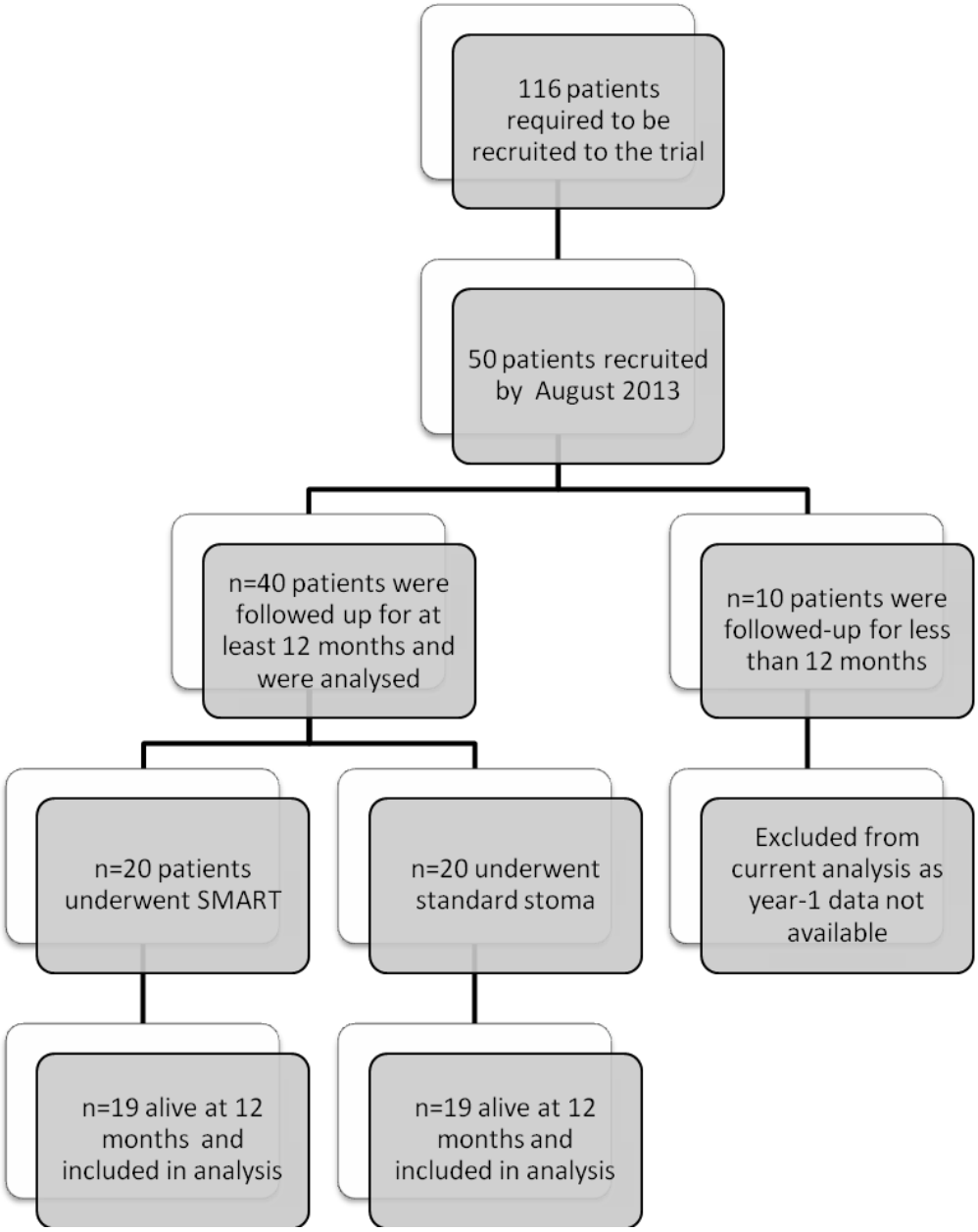


Table 4.1 Characteristics of patients with 12 month follow-up assessment completed

Parameter	**SMART Patients n=19**	**Standard stoma Patients n=19**	**p-value**
Age	65±11	71±13	0.27
Gender	6M:13F	11M: 8F	-
BMI	27±6	26±9	0.62
Preoperative albumin	39±8	39±7	1.0
ASA grade			
I	2	3	
II	8	9	
III	9	6	-
IV	0	1	
Indication for operation			
Malignancy	13	16	
Functional	5	0	
IBD	1	1	-
Diverticular Disease	0	1	
Pouch Failure	0	1	
Type of stoma			
Colostomy	15	16	
Ileostomy	4	3	-
Time taken for stoma (mins)	22±8	22±9	0.91

4.4 Discussion and conclusions

In this randomised controlled trial, preliminary results suggest that SMART reduces the incidence of parastomal herniation within the first postoperative year in patients who have undergone permanent stoma formation. The parastomal herniation rate following SMART is comparable to the rates reported previously following prophylactic mesh reinforcement of the stoma site (Chapter 1, Table 1.5) but direct comparison is not possible because of the different methodologies, selection criteria and mode of assessment. It should be emphasised, however, that patients were not excluded from this study if they were at high risk for herniation (e.g. obesity, respiratory disorders) and their mode of assessment included both clinical and radiological means in contrast to previous studies.[72 132]

The reduction in the herniation rate appears to be associated with improvements in quality of life on certain domains (e.g. mobility, overall score) of the EQ-5D questionnaire although it is difficult to attribute them exclusively to the reduced incidence of herniation because of the different pre-operative scores of patients in the control and test arms of the study.

Stoma-related morbidity did not differ between the treatment arms. One case of stoma necrosis occurred in the SMART group but it is likely to be related to the blood supply of the distal bowel segment being under tension rather than compression of the mesentery by the stapled trephine since the complication did not persist following resection of the affected segment. Additionally, there were no reported cases of mesh infection, fistulation or explantation in the SMART cohort during the observation period, providing further evidence that prophylactic mesh reinforcement is safe and should be routinely offered to all patients who undergo permanent stoma formation. This study has also contributed to

existing evidence that synthetic meshes such as Vypro® are safe and can be used to prevent parastomal herniation in preference to biological materials in view of their significantly lower cost.[158]

SMART did not appear to prolong the operating time and although technically more difficult than standard stoma formation, it was still graded as average or less than average difficulty in approximately two-thirds of cases. This figure may improve in the future as surgeons perform a greater number of SMART stomas and become more familiar with its technical aspects. Furthermore, the technique may be further simplified by gluing or stapling rather than suturing the mesh in the anterior rectus sheath with potential reduction in its actual operative time and greater surgeon acceptance. It can also be argued that, in view of its efficacy and ease of use in open or laparoscopic surgery, the procedure may become the "gold standard" for stoma formation.

It is believed that the superior efficacy of SMART in reducing the rate of parastomal herniation is due to the fact that it avoids creating an oversized defect, an independent risk factor for herniation, and simultaneously reinforces the abdominal wall, especially the stronger anterior rectus sheath, with mesh. All stomas were fashioned using circular staplers with knife diameters less than 25mm which results in the creation of a controlled, rigid, reinforced trephine that withholds its size and integrity with time. Nevertheless, the procedure carries the additional cost of £400 pounds per patient which is attributed to the use of the stapling device (£300) and the mesh (£100). This has to be interpreted in the context of a reduction in the incidence of parastomal herniation and avoidance of an open or laparoscopic operation for a symptomatic parastomal hernia which carries a much higher cost and the potential for morbidity (Chapter 1, section1.8).[174]

The encouraging results associated with SMART should be interpreted in the context of two important limitations. Firstly, the study was designed to recruit 116 patients and this preliminary analysis is based on 40 patients who completed their year one postoperative assessment. Thus, definitive conclusions require completion of recruitment and statistical validation. Secondly, all recruited patients will need to be followed up for at least 5 years to assess the long-term rates of infection, parastomal herniation and fistula formation in order to establish the true safety and efficacy of SMART and whether it should be routinely performed in all patients undergoing permanent stoma formation.

In conclusion, the 'SMART' technique refines and standardises stoma formation and reinforcement and addresses many technical limitations associated with manual stoma formation which may explain its current superiority in preventing parastomal herniation. Further long-term data of this RCT, however, will be needed to determine its true efficacy.

CHAPTER 5

An explorative study into the use of mechano-growth factor (MGF) as a biomarker for muscle injury.

5.1 Introduction

It is well known that striated muscles respond to mechanical stimuli with hypertrophy and increase in muscle mass. Muscle growth is under the influence of the growth hormone (GH)/insulin-like growth factor-1(IGF-1) axis. GH is produced by the pituitary and induces IGF-1 expression in the liver which is then released into the circulation, regulating systemic growth and development.[175] [176] However, mechanical stimulation of a particular muscle induces localised hypertrophy implying that there must be autocrine factors controlling growth and muscle phenotype. Animal experiments have shown that rapid hypertrophy of the tibialis anterior due to mechanical stimulation[177] is accompanied by a huge increase in mRNA indicating that muscle fibre hypertrophy may be controlled at the level of transcription with the increase in mRNA suggesting that more message is translated into protein.[178] Conversion of this mRNA product to cDNA and subsequent sequence analysis demonstrated it to be a splice variant of the IGF-1 gene but with a different sequence to the hepatic or systemic IGF-1 isoform (IGF-1Ea).[179] As this splice variant was expressed only in mechanically stimulated but not resting muscles it was termed mechano-growth factor (MGF).[180]

In vivo experiments have shown that intramuscular administration of the cDNA of both IGF-1Ea and MGF resulted in a 25% increase in muscle fibre size within 3 weeks.[175] However, injection of a viral construct containing the hepatic IGF-1 isoform (IGF-1Ea) produced a 15% increase in muscle mass in over four months.[181] The explanation for the rapid muscle hypertrophy following MGF administration is that the two isoforms are both important regulators of muscle mass but perform different functions. *In vitro* experiments involving muscle stem cell cultures treated with mature IGF-1 increased in mass and fused

to form myotubes. In contrast, treatment of muscle stem cultures with MGF increased the number of myoblasts which did not fuse but maintained their integrity as mononucleated cells.[182] It is now clear that MGF activates muscle stem cells which provide the extra nuclei for growth (i.e. muscle is a post mitotic tissue) and "kick starts" the hypertrophy process whereas the IGF-1Ea isoform is responsible for up-regulating protein synthesis. [175] Further evidence confirming the different roles of the two isoforms, MGF and IGF-1Ea, is provided by the distinctly different expression kinetics. Exercise and muscle damage induce initial splicing of the IGF-1 gene towards MGF but after a day the gene is almost completely spliced towards the IGF-1Ea isoform which then drives the anabolic process.[183 184]

The ability of muscles to respond to exercise and injury is age-dependent. Animal experiments showed that older muscles are more susceptible to injury and regenerate more slowly resulting in impaired functional recovery.[185] Muscle regeneration is dependent on the pool of stem cells to provide the extra nuclei for repair and growth but this pool is not adequately replenished in elderly muscles[186] which may be due to reduced ability to express MGF.[187] In particular, mechanical overloading of animal striated muscles resulted in over-expression of MGF mRNA which was three to five times higher in younger animals. IGF-1Ea mRNA levels were also up-regulated following mechanical stimulation but there was no age- related effect. In a similar human study, it was established that MGF and IGF-1Ea mRNA resting expression levels did not differ between young (25-36 years) and elderly (76-82years) subjects. However, mechanical overloading of the quadriceps femoris muscle resulted in significant increase in MGF mRNA expression in young but not elderly subjects within 2.5 hours.[176] Furthermore, mechanical overloading did not affect the

IGF-1Ea levels in the two groups confirming once again the differential regulation, roles and expression profiles of the two isoforms.

Age has been shown to be an independent risk factor for the development of herniation which may be related to intra-operative muscle damage and its ability to recover postoperatively.[188] This may explain why surgical techniques (i.e. lateral rectus abdominis positioned stomas) that preserve muscle integrity and avoid muscle fibre splitting and stretching reduce the incidence of para-stomal herniation even in the presence of other risk factors.[189] It is thought that the 'SMART' procedure minimises overstretching and muscle damage which contributes to its higher efficacy in preventing parastomal herniation.

Previous research has shown up-regulation of IGF 1Ea and MGF variants in the levator ani muscle following stretch injury after vaginal delivery. Cortes *et al* observed markedly up-regulated MGF (> 100-fold) and IGF-1Ea (>1000fold) levels in women within 1 hour of delivery compared to the baseline levels of control subjects. They concluded that damaged levator ani muscle results from stretch and overload after vaginal delivery.[190] We hypothesise that quantification of MGF and IGF-E1a expression of the rectus abdominis muscle will inform on the extent of the injury and the muscle's reparative ability in response to iatrogenic injury. This may provide the basis for novel therapeutic interventions including potential MGF replenishment therapy to prevent parastomal herniation. The proposed study using the clinical model of parastomal hernia could then be extrapolated to the wider field of prevention of incisional hernias.

The aim of this explorative study was to quantify rectus abdominis MGF and IGF-1Ea levels intra-operatively and assess the potential use of MGF as a biomarker for quantifying abdominal muscle injury.

5.2 Materials and Method

5.2.1 Patients

Participants in the SMART randomised controlled trial were also recruited to this study following fully informed consent. Only patients scheduled to undergo permanent stoma formation during open surgery were recruited because of the difficulty associated with obtaining laparoscopic biopsies of the rectus abdominis muscle and the variable effect of pneumoperitoneum which stretches the abdominal musculature. Stomas were fashioned using either the standard technique or the SMART technique depending on the outcome of the randomisation process (Chapter 4, section 4.2.2). Ethical approval was obtained by the National Research and Ethics Committee in the United Kingdom (NREC, West London REC 3, 10/H0706/92).

5.2.2 Power & sample size calculation

As this was a feasibility study a power calculation was not performed. It was decided to recruit a total of 10-15 consecutive patients of both genders to undergo intra-operative biopsy of the rectus abdominis muscle during open surgery.

5.2.3 Muscle biopsy procedure

Fully anaesthetised patients underwent random biopsy during laparotomy, using a 5mm punch biopsy needle (STIEFEL® biopsy punch), of the right and left rectus abdominis muscle at the start of the operation when the muscle became visible and just before closure

of the midline laparotomy wound. Random biopsies were similarly obtained during stoma formation, whether standard or SMART, from the rectus muscle immediately after opening of the anterior rectus sheath and before exteriorisation of the bowel. The biopsies were immediately submerged in RNA later stabilisation reagent (QIAGEN, Crawley, UK) to prevent RNA degradation and were stored in -80^{o} C within 24 hours.

5.2.4 Processing of samples

5.2.4.1 RNA extraction and quantification

Total RNA was extracted from the muscle samples according to the detailed protocol in Appendix II. The extracted RNA was dissolved in RNAse-free water and the concentration was determined spectrophotometrically using NanoDrop® (NanoDrop Technologies Inc, Delaware, USA) which measures sample absorbance at 260nm and 280nm wavelengths. Samples were measured twice with the mean absorbance reading at 260nm wavelength designated as the RNA concentration. RNA preparation quality was expressed by representing absorbance readings as a ratio, with reading taken at 260nm wavelength identified as numerator and reading at 280nm wavelength designated as the denominator. Preparation with ratios ranging from 1.8-2.1were only used for subsequent analysis with all other samples discarded.

5.2.4.2 cDNA synthesis using reverse transcriptase

RNA transcription into complimentary DNA was performed using the Omniscript reverse transcriptase kit (QIAGEN, Crawley, UK). To facilitate the efficiency of reverse

transcription in transcripts that were expressed at low levels, such as MGF, short sequence specific dodecamers 50 to 100 base pair downstream of the PCR reverse primers were used. The components required for the reverse transcription reaction and the protocol used are described in detail in Appendix III.

5.2.4.3 Generation of RT-PCR standards

PCR products for the MGF and IGF-1Ea genes were generated using the protocol described in Appendix IV. Following RT-PCR, PCR products were subject to agarose gel (2%) electrophoresis with a DNA ladder to confirm product size. PCR products were excised from the gel and purified using the QIA quick gel extraction kit (QIAGEN) according to the protocol in Appendix V. Based on the concentration of DNA obtained (measured using NanoDrop), the number of copies in a given volume was calculated and used to make serial dilutions of standards with known copy numbers of the gene of interest (Appendix VI).

5.2.4.4. Real time polymerase chain reaction (RT-qPCR)

In this step, complimentary DNA is amplified and quantified using multiplexed gene specific primers. Target specific primers for MGF and IGF-1Ea as well as three reference "housing-keeping" genes GAPDH, B2M (beta-2-microglobulin) and B-actin that have been shown previously to be stable under the chosen experimental conditions in our laboratory were used. Primer sequences (Table 5.1) were exactly the same as the ones used by Cortes *et al* previously to quantify MGF and IGF-1Ea expression.[190] All primers were synthesized by Sigma-Aldrich (Sigma-Aldrich Ltd, Dorset, UK).

Quantification of mRNA coding for IGF-1Ea, MGF, GAPDH, B2M and B-actin was performed with RotorGene 6000 (Qiagen, UK) using SYBR green I (Qiagen, UK), a dye that binds to the minor groove of double-stranded DNA. Quantitative PCR was performed in a total reaction volume of 20μl containing 300 μM forward primer, 300 μM reverse primer, 10 μl SYBR Green and 4ul standard/100 ng cDNA template. Reaction conditions were as follows: initial activation at 95 ^{o}C for 15 minutes, denaturation at 95 ^{o}C for 20 seconds, annealing at 60 ^{o}C for 20 seconds, extension at 72 ^{o}C for 20 seconds and final extension at 72 ^{o}C for 15 seconds. Reactions were run for 30-40 cycles. Rotor Gene 6000 series software 1.7 was used for analysis of copy number in unknown samples. On completion of PCR, melt curve analysis was performed to confirm the presence of one single product, with all PCR products sequenced to confirm identity (Figure 5.1). Test samples were run in duplicate in parallel with cDNA standards of known gene copy number and negative controls (no template and no enzyme). Cycle threshold (CT) values were used for analysis, and actual gene expression in test samples was quantified using the generated standard curve (Figure 5.2). All samples of unknown concentration fell within the dynamic range of the standard curve. Data for the genes coding for MGF and IGF-1Ea were then expressed relative to a normalisation factor generated from a panel of 3 housekeepers (GAPDH, β-actin and β-2 microglobulin) using a commerically available software (GenNorm, Biogazelle, Version 3.5, Belgium). Runs were performed in duplicate and mean values were subsequently used for analysis.

Table 5.1 Primer sequences used in real-time PCR

Primer Name	Sequence (5' to 3')	Product Size (bp)	Accession no
GAPDH	Forward: GGAAGCTTGTCATCAATGGAA Reverse: TGGACTCCACGACGTACTCA	102	NM_002046.3
B-**actin**	Forward: CCAACCGCGAGAAGATGA Reverse: CCAGAGGCGTACAGGGATAG	97	NM_001101.2
B2M	Forward: TAGGAGGGCTGGCAACTTAG Reverse: CTTATGCACGCTTAACTATCTTAACAA	127	NM_004048.2
IGF-1Ea	Forward: GCCTGCTCACCTTCACCAGC Reverse: TCAAATGTACTTCCTTCTGGGTCTTG	303	U40870
IGF-1Ec (MGF)	Forward: CGAAGTCTCAGAGAAGGAAAGG Reverse: ACAGGTAACTCGTGCAGAGC	150	X57025

Figure 5.1 Melting curve profile corresponding to a single band of the predicted size for (A) MGF and (B) IGF-1Ea.

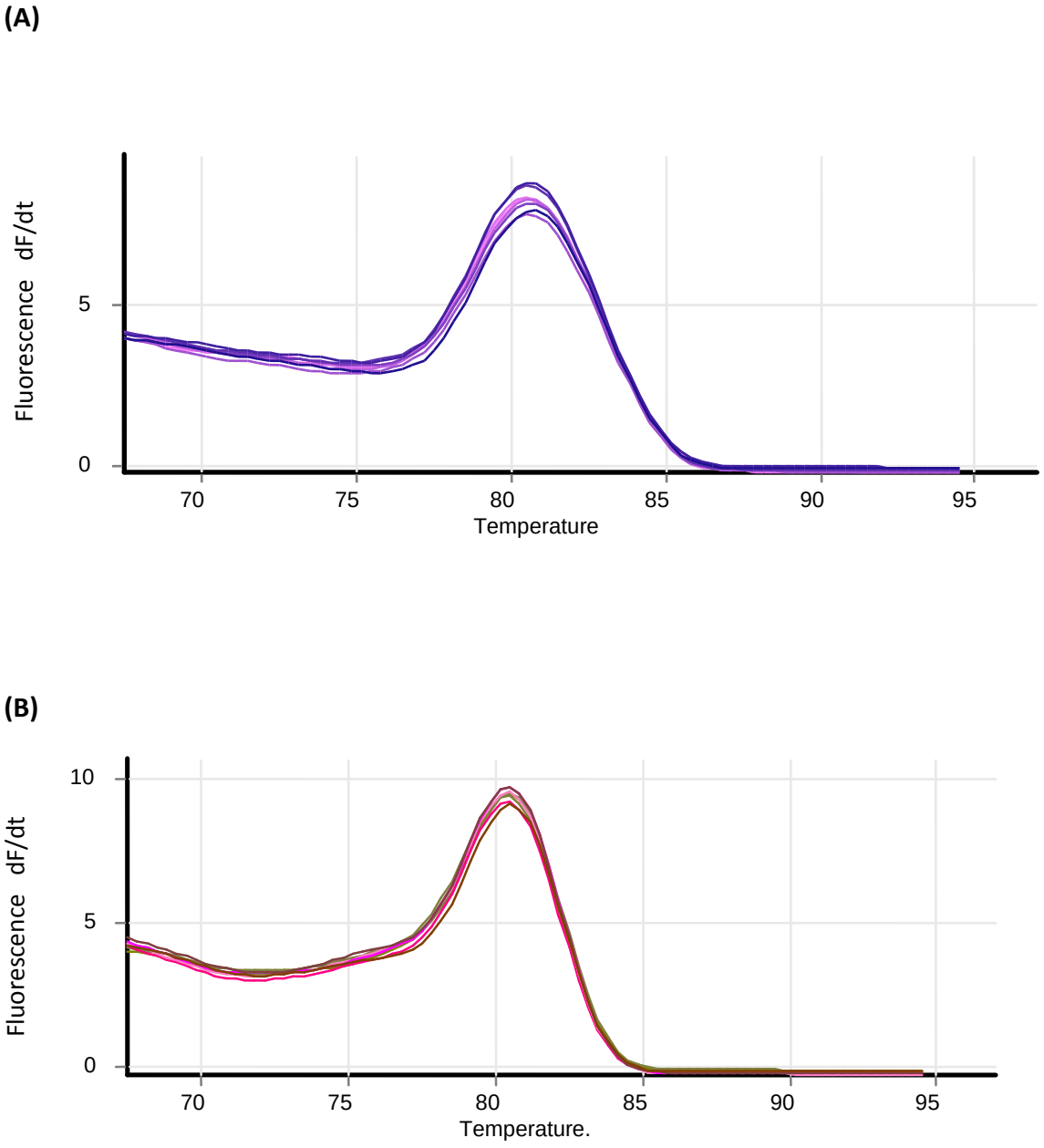
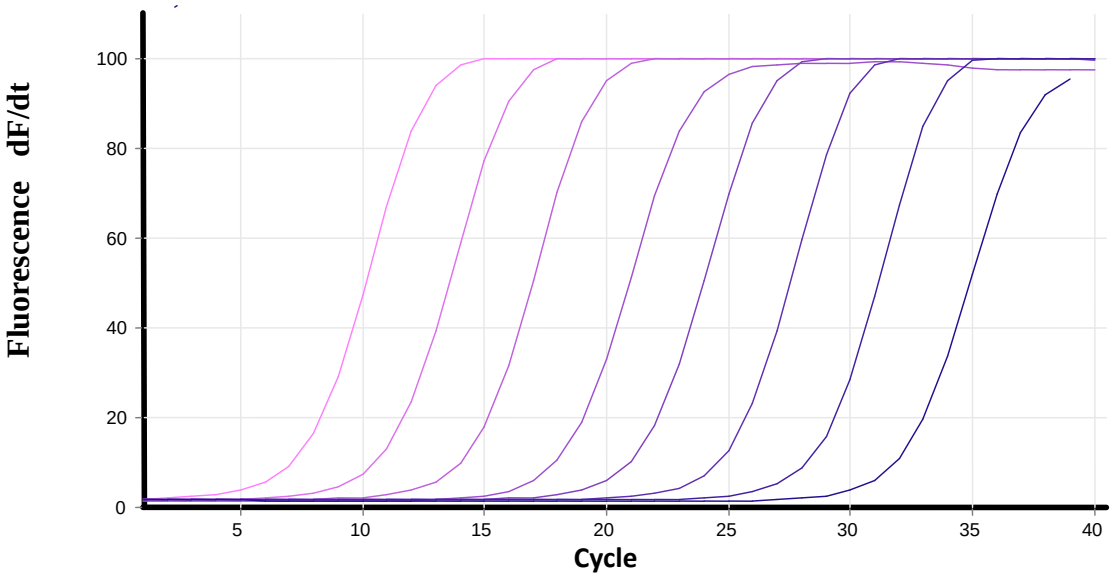
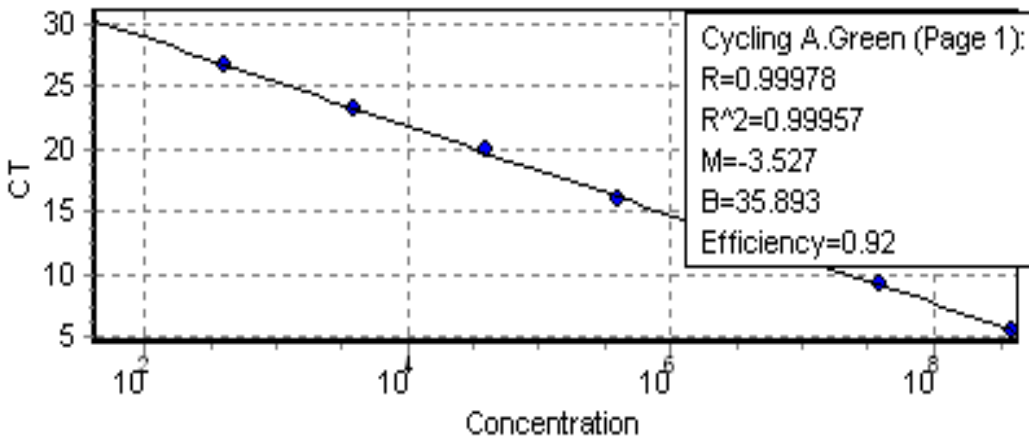


Figure 5.2. (A) Amplification profile of standards of known concentrations of MGF DNA. (B) the standard curve generated by the Rotor Gene software. For each sample the crossing point was plotted against the known concentration of the standard. The resulting curve is shown as a graph of cycle number vs log concentration.

(A)



(B)



5.3 Data analysis

The data were tabulated on an Excel Spreadsheet. Data normality was assessed using a commercially available software package (GraphPad Version 5, GraphPad Software Inc, La Jolla, CA). Data normality was assessed using the De Agostino-Pearson omnibus normality test. Normally distributed data have been presented using mean and standard deviation, whereas non-normal data have been presented as a median and range. Normally distributed data were compared using paired t-tests. Analysis of non-normal data was performed using the Mann-Whitney U tests**.** The level of statistical significance was taken as <0.05.

5.4 Results

There were 13 patients (6M: 7F) with a median age of 77 years (range, 40-81) who underwent open biopsies of the rectum abdominis muscle during laparotomy and stoma formation (6 SMART and 7 standard).

5.4.1 MGF expression levels

The median baseline (i.e. start of laparotomy) MGF expression level of the right rectus abdominis muscle was 309 (range 184-560) and did not differ statistically to the baseline MGF expression of the left rectus abdominis muscle (median 162, 113-7195; p=0.4). Just before closure of the laparotomy wound, the MGF levels of the right (median 288, range 158-5160) and left (median 180, range 70-544) rectus abdominis muscles were again comparable (p=0.3). Comparison of the MGF expression levels for each muscle between the start and end of operation did not reveal any statistical difference (right rectus p=0.5; left rectus p=0.3). No statistical difference in the expression levels was seen between men

and women for either the right or left rectus muscle at the start or the end of the operation. The effect on age on the MGF expression could not be established as all patients, but one (age 40), were over the age of 70.

The median MGF level before stoma formation (as the anterior rectus sheath was opened) was 305 (126-3485) and did not differ to the level after stoma formation (188, 136-1045; p=0.3) or with the surgical technique used (SMART versus standard; p=0.6)

5.4.2 IGF-1Ea expression levels

The baseline IGF-1Ea level for the right rectus muscle was 3438 (626-11081) and was not different to its expression level just before closure of the laparotomy wound (median 2621, range 222-11524; p=0.81). Similarly, there was no difference in IGF-1Ea levels between start (median 1022, range 564-18298) and end of the laparotomy (median 1878, range 187.6-15987; p=0.81) for the left rectus muscle. No difference in the expression levels was seen between the two muscles at the above time points (p>0.5). No statistical difference in the expression levels was seen between men and women for either muscle.

The IGF-1Ea level before stoma formation (median 761, range 566-7234) was not statistically different to the level after stoma formation (median 791, range 500-6236; p=0.17) and did not differ with surgical technique (SMART versus standard, p=0.67).

5.5 Discussion and conclusions

In this pilot study, the MGF and IGF-1Ea expression levels did not appear to change intra-operatively. Levels were similar for both men and women and were not influenced by the surgical technique used to fashion the abdominal stoma. IGF-E1a appeared to be expressed approximately ten times more than MGF which is consistent with the results of a previous study.[176] This is thought to reflect its hepatic synthesis and release into the systemic circulation.[190]

The failure of this study to demonstrate any change in the intra-operative expression of MGF and IGF-1Ea may be due to the small sample size introducing a type II error. However, the pharmacokinetic profiles of the two gene products and the age of the study participants are two parameters that require further discussion. Using an animal model to study the MGF and IGF-1Ea expression kinetics in chemically and mechanically damaged striated muscle, Hill and Goldspink showed that MGF levels peak within one day while IGF-1Ea reaches its peak on day 11 when the MGF expression has progressively declined.[183] The different expression profiles of the two gene products reflect their different function with MGF causing rapid proliferation of myotubes and IGF-1Ea enhanced terminal differentiation and fusion of satellite cells with the damaged muscle fibres in order to kick-start muscle repair and regeneration.[182] Consequently, the relatively short time period between the initial and repeat biopsy might be an important contributing factor since the duration of a laparotomy for a colorectal operation is approximately 3-4 hours which may not be enough to allow for the increased gene expression to be detected. Nevertheless, Hameed *et al* reported increased MGF expression between 2% and 864% of the quadriceps femoris muscle within 2.5 hours following mechanical stimulation. The

increase was only observed in young healthy men of approximately 30 years of age while older subjects (mean age of 75 years) did not demonstrate any change in the MGF expression. In the same study, the baseline and post- mechanical stimulation levels of IGF-1Ea were not different between younger and older subjects.[176] The median patient age in this cohort was 77 years and this may be another contributory factor explaining the lack of differential expression.

Despite the negative findings of this preliminary study, the results should be taken into consideration when designing similar future studies exploring the use of MGF as biomarker for abdominal muscle injury and repair. In particular, increasing the interval between the initial and repeat biopsy, in order to establish whether a prolonged time period allows for increased gene expression to be measured, seems appropriate. MGF expression peaks at 24 hours with increased IGF-Ea expression thereafter but performing serial abdominal muscle biopsies in the early postoperative period is not possible as most patients will not return to the operating room, unless clinically required. Furthermore, there are valid ethical concerns associated with recruiting high-risk postoperative patients in an experimental study. While the ideal study may not be possible due to the aforementioned reasons, an alternative study may want to recruit patients who undergo temporary ileostomy formation for benign or malignant intra-abdominal pathology. In this scenario, the initial biopsy can be performed during ileostomy formation with the repeat biopsy at 3-6 months when the patient is scheduled to undergo ileostomy closure. This can also allow recruitment of younger patients who undergo stoma formation for inflammatory bowel disease, thus allowing the effect of age on MGF and IGF-E1a expression to be studied. Recruiting paediatric patients undergoing major abdominal surgery and stoma formation is another interesting potential

cohort in which peri-operative MGF should be assessed in view of the greater ability of younger muscles to regenerate.[185]

In conclusion, this study has not demonstrated a role for MGF or IGF-E1a in abdominal muscle injury and repair. The feasibility of performing a methodologically improved study is in doubt because of the pharmacokinetic profiles of these gene products and the ethical problems associated with subjecting postoperative surgical patients into serial abdominal wall biopsies. An animal model should be used in the first instance in order to establish whether these products can be used as diagnostic and therapeutic means for incisional and parastomal hernias.

CHAPTER 6

A radiological assessment of rectus abdominis muscle in parastomal herniation

6.1 Introduction

The mechanism of wound separation and hernia formation is multi-factorial and involves chemical and mechanical pathways (please see Chapter 1, section 1.2). Chemical mechanisms involve collagen synthesis and deposition with the local wound healing environment also being influenced by factors such as ischemia and infection.[191] The abdominal wall, however, is a muscular structure under a dynamic equilibrium of forces which are disturbed following laparotomy and stoma formation. In particular, when a midline incisional hernia develops, the abdominal wall muscles express a pattern of changes which are characterised by atrophy.[23]

There is currently no evidence regarding the relationship between muscle structure or size and parastomal hernia formation. Objectively assessing the structure or function of the abdominal wall may provide improved means of risk stratification and guide on different management strategies for patients with different physiological characteristics. It is standard practice for patients with colorectal malignancy to undergo computed tomography (CT) imaging to assess the extent of the disease and plan their surgical treatment. Routine cross-sectional images, however, may also inform on the abdominal muscle size and content and its contribution to the development of parastomal herniation. CT is a non-invasive technique that is considered one of the criterion measures for assessing skeletal muscle mass.[192] Previous studies showed that CT images taken at T12-L1 and L4-L5 inter-vertebral axis can be used to assess skeletal muscle mass and density.[192 193] Furthermore, in view of the non-invasive nature of imaging and its invariable availability prior to any major surgical intervention, investigators have explored its ability to assess muscle size and its relation to age and surgical outcomes.[194 195]

CT has the capability to distinguish between different tissues in vivo on the basis of their attenuation characteristics which are related to tissue density and composition.[196] CT based attenuation values are expressed in Hounsfield units (HU) on the basis of a linear scale using water as the reference (0 HU). In particular, CT can distinguish adipose tissue which has a negative attenuation value whereas muscle attenuation is positive. The detailed spatial assessment of attenuation coefficients provided by CT can be used to assess tissue areas with a specific range of attenuation values and the mean tissue attenuation. Previous studies using CT to assess muscle composition have reported an association between reduced skeletal muscle attenuation and diminished muscular strength.[197] Further studies have also shown that weight loss increases the mean attenuation value of muscle which is related to its lipid content.[196 198]

The aim of this study was to radiologically quantify the preoperative and postoperative structure of the rectus abdominis (RA) muscle and establish whether structural changes are related to the development of parastomal herniation.

6.2 Materials and Method

6.2.1 Radiological assessment

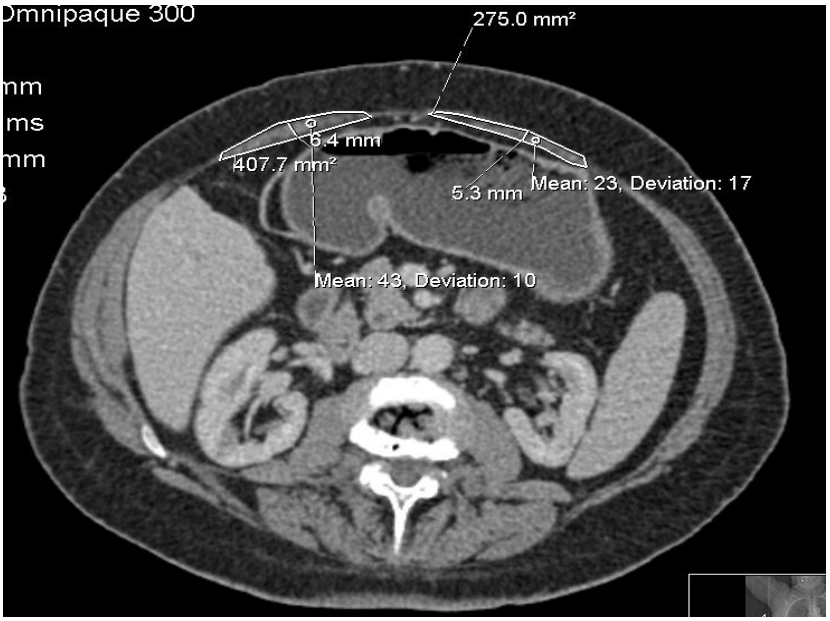
Pre-operative and postoperative 5mm cross-sectional abdominal images obtained using an electron beam CT by standard protocol following intravenous contrast administration were examined. Measured variables included: rectus abdominis (RA) cross-sectional area

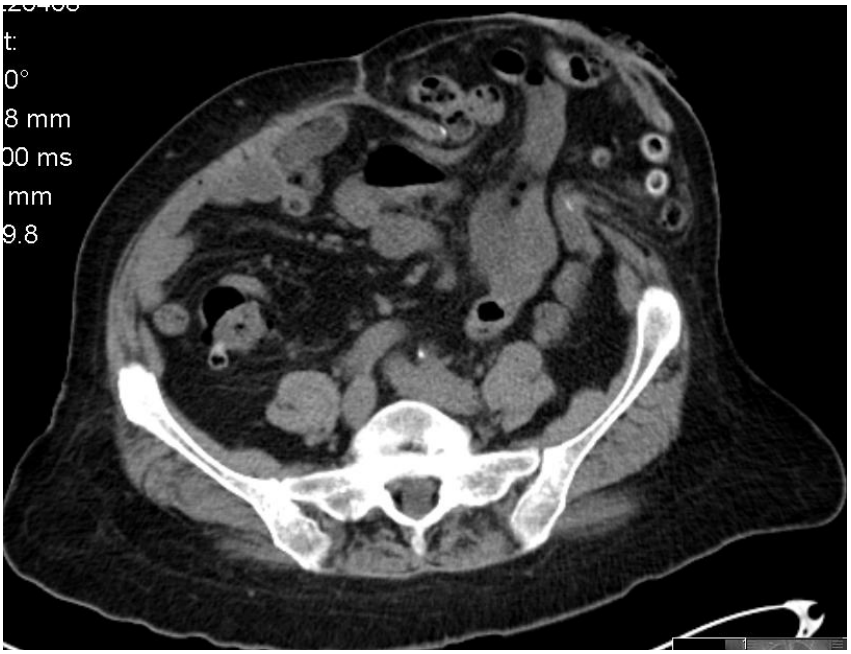
(CSA), maximum thickness and maximum density in Hounsfield units. All parameters were measured at the mid inter-vertebral horizontal axis at the T12-L1 and L2-L3 levels. The CSA of the right and left RA muscle was measured by outlining its borders from the lateral aponeurotic attachment to the linea alba and calculating the enclosed area and maximum muscle density (Fig. 6.1). Maximum muscle thickness was measured as the distance between the deep and superficial fascia at the widest distance of the enclosed area. All these steps were performed in a semi-automated fashion using algorithms programmed in the computer system of our Radiology department (SECTRA PACS IDS 5; version 11.4, 2009; IMTEC AD, Sweden).

Rectus abdominis muscle reconstruction was also performed by using all 5mm cross sectional images, as described previously, from the xiphisternum to the pubic symphysis. Total muscle volume and mean density were calculated using a commercially available software (OsiriX MD, Pixmeo SARL, Switzerland).

All measurements were performed by two researchers and an average value was used for the final analysis.

Figure 6.1 Illustration of radiological assessment of rectus abdominis muscle in a patient who developed a large parastomal hernia.





6.2.2 Patients

All patients who underwent permanent stoma formation in our institution (i.e. Barts' Health NHS Trust) as part of the previously described SMART randomised controlled trial (Chapter 4) were included in the study. The right and left rectus muscle was assessed at baseline (using the preoperative CT scan) and postoperatively at one year. The scans were performed according to the SMART trial protocol to detect a parastomal hernia (Chapter 4, section 4.2). Patients who were recruited in other centres were excluded from this study because their preoperative and postoperative radiological assessment was performed using different equipment, dose of contrast and imaging protocols. Data collected prospectively included patient demographics (age, gender, body-mass index [BMI]) and radiological evidence of parastomal herniation .

6.2.3 Statistical analysis

The data were tabulated on an Excel Spreadsheet. Data normality was assessed using a commercially available software package (GraphPad Version 5, GraphPad Software Inc, La Jolla, CA). Data normality was assessed using the De Agostino-Pearson omnibus normality test. Normally distributed data have been presented using mean and standard deviation, whereas non-normal data have been presented as a median and range. Normally distributed data were compared using paired t-tests. Analysis of non-normal data was performed using the Mann-Whitney U tests**.** A p value of <0.05 was considered statistically significant

6.3 Results

There were 20 patients (14F: 6M) with a median age of 70 years (range 40-84) who underwent permanent stoma formation (colostomy n= 18 or ileostomy n= 2) in open (n=18) or laparoscopic surgery (n=2). The majority of patients were operated for colorectal malignancy (n=18, 90%) with the rest requiring a permanent stoma for inflammatory bowel disease (n=1, 5%) or a functional bowel disorder (n=1, 5%). Preoperative and postoperative abdominal CT scans were available for all of them.

6.3.1 Preoperative and postoperative muscle radiological assessment

T12-L1 Level

The pre-operative CSA (median 300.3 mm^2, range 123.3-774.4) of the rectus muscle on the stoma site was not different from the CSA on the non-stoma (control) side (median 322.4 mm^2, range 112.6-840.8; p=0.8). Postoperatively, the CSA on the stoma (199.3mm^2, range 109-605.3, p=0.1) and non-stoma (258.4 mm^2, range 140.0-563.6, p=0.1) sides appeared reduced but comparable to one another [p=0.2, (Table 6.1)]. Muscle thickness on the stoma side was statistically reduced at the one year assessment compared to baseline (5.5 mm versus 6.5 mm, p=0.04) but muscle density appeared unchanged. Preoperative and postoperative muscle thickness and density for the non-stoma side were also statistically comparable (Table 6.1).

L2-L3 Level

The CSA and thickness of the rectus muscle on the stoma side decreased postoperatively compared to baseline but the difference was not statistically significant (Table 6.1). The peri-stomal muscle density appeared increased but, once again the effect did not attain statistical significance. All three studied variables appeared unchanged for the rectus muscle on the non-stoma side (Table 6.1). There was no statistical difference in the preoperative and postoperative values of the three parameters for the stoma and non-stoma sides.

Muscle reconstruction

The preoperative muscle volume did not differ between the stoma (91.0±44.2 cm^3) and non-stoma sides (94.8±47.2cm^3; p=0.8). Postoperatively, muscle volumes were comparable (p=0.1) between the stoma (89.6±36.8 cm^3; p=0.7) and non-stoma sides (90.4±40.1cm^3; p=0.4) without any change from their baseline values (p=0.1).

Mean preoperative muscle density was not statistically different (p=0.8) between the stoma (20.7±17.7 Hounsfield units) and non-stoma sides (18.7±22.4 Hounsfield units). Postoperative muscle density appeared reduced for both muscles [(stoma side 16.9±17.5 Hounsfield units; p=0.3);(non-stoma side 18.4±21.5 Hounsfield units)] but not statistically different when compared to baseline values or to each other (p=0.1).

Table 6.1. Preoperative and postoperative radiological muscle assessment

Parameter	Preoperative	Postoperative	p-value
T12-L1 Level			
CSA(stoma)	300.3 (123.0-774.4)	199.3(109.7-605.3)	0.1
CSA(non-stoma)	322.4 (112.6-840.8)	258.4(140.0-563.6)	0.1
Thickness(stoma)	6.5 (6.0-10.2)	5.5 (3.4-8.2)	**0.04**
Thickness(non-stoma)	6.0 (5.0-11.0)	6.5 (3.5-10.4)	0.7
Density (stoma)	47 (14-56)	45(10-62)	0.8
Density (non-stoma)	40 (20-67)	44(14-76)	0.8
L2-L3 Level			
CSA(stoma)	327.7 (125.3-614.8)	264.2(136.6-520.2)	0.3
CSA(non-stoma)	307.7 (125.5-633.4)	349.5(153.2-543.0)	0.8
Thickness(stoma)	6.1 (3.1-13.0)	5.3 (3.4-11.6)	0.5
Thickness(non-stoma)	6.3 (3.0-13.0)	6.3 (4.0-11.6)	0.8
Density (stoma)	25 (-62-54)	47(-1-74)	0.1
Density (non-stoma)	39 (-44-55)	43(-29-70)	0.3

6.3.2 Preoperative and postoperative muscle radiological muscle assessment of patients with parastomal hernia versus patients without herniation.

There were 7 patients (age 67±15years, BMI 27±8kg/m^2) who were diagnosed radiologically with a parastomal hernia while 13 (age 63±14years, BMI 29±10kg/m^2) did not have any evidence on CT assessment. Age (p=0.3) and BMI (p=0.5) were similar for the two groups.

T12-L1 Level

Muscle assessment at T12-L1 revealed that all measurable parameters were reduced postoperatively compared to baseline for patients with herniation but the differences were not statistically significant (Table 6.2). Patients without parastomal herniation also demonstrated reduced CSA and thickness compared to baseline but the effect was again not statistically significant (Table 6.2) Intergroup comparison revealed similar preoperative CSA (292.3 versus 282.0 mm^2, p=0.9), muscle thickness (6.0 versus 6.5mm, p=0.5) and density (29 versus 49 Hounsfield units, p=0.5) between hernia and non-hernia patients. However, post-operatively muscle density was significantly reduced in patients with herniation compared to patients without a hernia (23 versus 53 Hounsfield units, p=0.04;Table 6.2). A similar effect was observed for muscle thickness with hernia patients having a thinner muscle on the stoma side (4.7 versus 6.0mm, p=0.3). Postoperative CSA, however, appeared similar for hernia and non-hernia patients (217.5 versus 199.3mm^2, p=0.7)

L2-L3 Level

Radiological assessment at L2-L3 showed that preoperative and postoperative comparison of all studied variables did not reveal any change in patients with or without herniation (Table 6.3). Similarly, the preoperative muscle CSA (250.6 versus 327.7mm^2, *p*=0.6), muscle thickness (5.7 versus 6.0mm, *p*=0.9) and density (5 versus 25 Hounsfield units, *p*=0.47) were all higher in patients without herniation but the difference was not statistically significant. Postoperatively, muscle density was lower in patients with herniation (median 11 Hounsfield units, range -1-38) compared to patients without herniation (median 49 Hounsfield units, range 34-74; *p*=0.006). Postoperative CSA (269.6 versus 231.4mm^2, *p*=0.7) and thickness (5.6 versus 5.0mm, *p*= 0.9) were not statistically different between the two groups.

Muscle Reconstruction

No difference was seen in the preoperative (86.0±47.2 cm^3) and postoperative (86.4±38.1 cm^3; *p*=0.9) muscle volume of the stoma side in patients without herniation. Muscle volume on the stoma side in patients with parastomal herniation was 82.7±27.1 cm^3 and remain unchanged postoperatively (78.8±21.0 cm^3; *p*=0.4). Comparison of the preoperative (p=0.1) and postoperative muscle (p=0.1) volumes of patients with and without herniation did not reveal any statistical difference.

Mean muscle density on the stoma side in patients without herniation appeared reduced postoperatively (21.2±18.2 Hounsfield units) but unchanged compared to baseline (25.6±12.7 Hounsfield units; *p*=0.4). Patients with parastomal herniation had a significantly lower muscle density preoperatively (1.9±19.7 Hounsfield units) and postoperatively

1.7±7.3 Hounsfield units; p=0.5) but no statistical difference was found when compared to the preoperative (p=0.1) and postoperative values (p=0.1) in patients without herniation.

Table 6.2. Relation between muscle and parastomal hernia development at T12-L1

Parameter	Preoperative	Postoperative	p-value
Patients with herniation			
CSA(stoma)	292.3 (233.4-415.5)	217.5(135.7-250.5)	0.2
Thickness(stoma)	6.0 (6.0-8.0)	4.7 (3.4-7.2)	0.2
Density (stoma)	29 (21-55)	23(10-45)	0.7
Patients without herniation			
CSA(stoma)	282.0 (123.3-774.4)	199.3(109.7-605.3)	0.5
Thickness(stoma)	6.5 (6.0-10.2)	6.0 (3.8-8.2)	0.2
Density (stoma)	49 (14-56)	53(25-62)	0.7

Table 6.3. Relation between muscle and parastomal hernia development at L2-L3

Parameter	Preoperative	Postoperative	p-value
Patients with herniation			
CSA(stoma)	250.6 (174.1-467.5)	269.6(136.6-353.1)	1.0
Thickness(stoma)	5.7 (4.0-7.0)	5.6 (3.4-5.9)	0.9
Density (stoma)	5 (-32-45)	11(-1-38)	1.0
Patients without herniation			
CSA(stoma)	327.7 (125.3-614.8)	231.4(142.2-520.2)	0.5
Thickness(stoma)	6.0 (3.1-13.0)	5.0 (4.0-11.6)	0.9
Density (stoma)	25 (-62-54)	49(34-74)	0.07

6.4 Discussion and conclusions

This radiological study represents the first attempt, to our knowledge, to assess the structure of the abdominal wall musculature peri-operatively and investigate the relationship between rectus abdominis and parastomal hernia development. The study has revealed that cross-sectional area, maximum thickness and maximum density decline postoperatively although the differences were not statistical significant . Furthermore, muscle reconstruction did not demonstrate any change in muscle volume postoperatively for the rectus muscle on the stoma or non-stoma side. Mean muscle density appeared to decline postoperatively with a greater decrease seen for the rectus muscle on the stoma side although the change did not become statistically significant.

A sub-analysis performed for patients with and without herniation showed that at the T12-L1 inter-vertebral axis the cross-sectional area and maximum thickness of the rectus muscle on the stoma side decreases postoperatively, albeit not statistically, for both hernia and non-hernia patients. However, maximum muscle density on the stoma side decreased slightly for patients with hernia and increased marginally in patients without herniation although in both cases the result was not statistically significant. Nevertheless, the postoperative maximum muscle density of the stoma site in patients without herniation was statistically higher compared to patients with a parastomal hernia. The picture was less clear at the L2-L3 assessment axis but the density of the rectus on the stoma side was again statistically higher in patients without parastomal herniation compared to patients with a hernia ($p=0.03$). Muscle reconstruction did not reveal any difference in the preoperative or postoperative muscle volume between patients with and without herniation but patients

with hernia had a significantly lower mean muscle density than those without parastomal herniation.

The findings of this study are in agreement with the results of previous studies which reported postoperative abdominal muscle changes associated with decreased cross-sectional area, atrophy and degeneration.[23 26 199] Our study has contributed to current evidence by demonstrating that the RA density is lower in patients with a parastomal hernia than patients without herniation. This is most likely due to postoperative infiltration of the muscle by adipose tissue. The reasons why certain patients have less dense abdominal musculature are currently unknown and may be related to patient age, gender and lifestyle but a formal multivariate analysis has not performed in view of the small sample size.

Unlike standard risk factors for parastomal hernia development (e. g. asthma, steroids, connective tissue disorders) muscle quality may represent and important parameter to identify patients who may benefit from a specific preoperative or postoperative intervention such as an exercise regime to improve abdominal muscle cross-sectional area, thickness and density.[200] Several exercise modes (e.g. pilates, swimming) have been shown to elicit abdominal muscle hypertrophy and have been recommended as an effective method to reinforce the abdominal wall and compensate for any pre-existing asummetric developments.[200 201] An exercise programme targeting the abdominal muscles of patients who undergo laparotomy also carries the potential benefit of improving their general physiological status, assisting respiratory rehabilitation and facilitating recovery since frailty and sarcopenia are associated with increased morbidity and mortality after major abdominal surgery.[194 202] An alternative strategy may involve postoperative implantation of synthetic or biological scaffolds seeded with myoblasts which have been found in

experimental studies to promote skeletal muscle regeneration.[203 204] One potential problem with such materials is that muscle growth factors and myoblasts may promote carcinogenesis and their use in patients undergoing stoma formation for colorectal malignancy is controversial.

There are some important limitations of this work. Firstly, the study sample is small which may introduce a type II error. This may explain why, despite a reduction in all measurable variables, statistical significance was not attained. Secondly, the follow-up period is limited to one year. A longer assessment interval would be ideal as not all hernias will have developed during the first postoperative year. Finally, the study included only patients who underwent preoperative and postoperative assessment at one institution (i.e. The Royal London Hospital) by a single consultant radiologist. Thus, a degree of measurement or expectation bias cannot be excluded. This can be minimised by performing the study in other institutions which use different protocols of computed tomography with the scans being interpreted by more than one radiologist and establishing the level of inter-observer variability.

In conclusion, the RA muscle density appears to decrease following major laparotomy and stoma formation with patients who develop a parastomal hernia having a much lower abdominal wall density postoperatively. Decreased postoperative muscle density may be an important remediable risk factor for the prevention of parastomal herniation. It is possible that in time, imaging may be used to predict the risk of incisional or parastomal hernia. The effect of a pre-operative or post-operative exercise regime on the abdominal musculature and the development of herniation warrants further investigation.

CHAPTER 7

Discussion of this thesis and proposals for future work

Parastomal herniation has been a major surgical problem for many decades but its impact on quality of life and other socioeconomic issues has only been recognised more recently. The challenge for surgeons in the coming years is to appreciate the current status of the problem and acknowledge the need for change and improvement in order to reduce the unacceptably high incidence of herniation and avoid the continuing unsatisfactory outcomes associated with its repair.

A comprehensive review of the literature performed in Chapter 1 showed that trans-peritoneal stoma formation through the rectus muscle is safe but it is associated with clinical or radiological parastomal herniation rates in excess of 50%.[173 174] There is some evidence, mainly level III and IV, that alternative techniques such as extra-peritoneal stoma formation or the lateral rectus abdominis positioned stoma (LRAPS) may reduce the parastomal herniation rate. A formal recommendation, however, on the optimum technique for stoma formation cannot be made due to the lack of Level I evidence. Large multinational randomised trials assessing the comparative efficacy of all techniques (e.g. extraperitoneal, LRAPS, circular devices) are urgently needed. Similarly, the literature review showed that there is insufficient evidence to advocate one technique for the repair of symptomatic parastomal hernias.[27 174] The actual approach depends on surgeon experience, available resources and patient's circumstances. Nevertheless, open or laparoscopic stoma reconstruction and reinforcement with a synthetic mesh seems a reasonable approach as the new synthetic meshes are improved in terms of biocompatibility and infectious complications and do not appear to be inferior to biological ones which are significantly more expensive. Special consideration should be given to the optimal anatomical plane for

mesh implantation. Future studies will need to compare the efficacy and safety of on-lay, sub-lay or intra-peritoneal mesh stoma reinforcement.

At this moment in time, it is the author's opinion that all patients, especially those at high risk (e.g. age> 60 years[205], malignancy[173]), scheduled to undergo permanent stoma formation should be offered the option of pre-peritoneal or sublay mesh reinforcement since previously conducted RCTs, despite their limitations, have reported encouraging results without any increase in stoma-related morbidity[174]. In fact, universal stoma reinforcement is now routinely performed in some Northern European countries.

Manual pre-peritoneal or sublay mesh implantation, however, is not routinely performed in the United Kingdom as it is thought to increase the operative time and can be particularly difficult if the stoma is constructed laparoscopically. Furthermore, an oversized trephine diameter > 25mm is, as reported in Chapter 2, a potential risk factor for herniation. The newly described Stapled Mesh stoma Reinforcement Technique (SMART) attempts to control the size of the trephine by using circular stapling devices which create a trephine< 25mm and simplifies mesh stoma reinforcement in open or laparoscopic surgery. The initial encouraging results in a highly selected patient cohort (Chapter 3) and in a sample of patients recruited to a large randomised controlled trial (Chapter 4) show that it reduces the parastomal herniation rate to approximately 15-20%. Completion of recruitment, however, and statistical validation the results are required prior to recommending routine use of SMART outside the boundaries of an RCT. Furthermore, the cost-effectiveness of procedure is currently unknown and the additional cost of approximately £400 for the stapler and the synthetic mesh may be prohibitive in the current climate of limited health care resources although it can potentially be "off-set" by a reduction in parastomal

herniation rates and cost of subsequent operations for symptomatic parastomal hernias. Thus, the long-term results of the trial are eagerly awaited.

The thesis also attempted to establish the contribution of the rectus abdominis muscle in the development of herniation using biochemical and radiological means. Investigation of the expression of a novel biomarker (i.e. Mechano-Growth Factor, MGF; Chapter 5), which was found in previous studies to correlate with muscle injury and repair, did not show a differential expression intra-operatively. This may well be due to the relatively short operative time of a colorectal procedure not allowing quantitive changes to be detected or due to loss of muscle ability to regenerate following injury, especially since the median age of patients who underwent intra-operative biopsy was 77 years and muscle regeneration and repair decline with age.[185 190] The latter explanation is a real possibility since radiological assessment of the rectus abdominis muscle showed a decrease in its thickness and density postoperatively with patients without herniation having a higher postoperative muscle density compared to patients with a parastomal hernia. This suggests that modification of the physiological characteristics of the abdominal musculature to improve its size and density may help reduce parastomal hernia development.

Future research needs to address the aetiopathogenesis of parastomal hernia formation as until we have fully understood the mechanism of its formation, direct prevention and treatment will always be unsatisfactory. Two areas are likely to attract further attention as they have potential therapeutic implications. Firstly, identifying genetic or environmental factors that affect collagen metabolism since parastomal hernias, and indeed all abdominal wall hernias, may represent the end-point of a condition characterised by a shift in collagen ratio from the strong type I to the "immature" type III.[11 12] Implantation of biomaterials

(e.g. mesh releasing Type I collagen), local administration of pharmacological adjuncts or gene therapy to re-adjust any imbalances in collagen metabolism may be future treatment strategies. Secondly, addressing the mechanics of the abdominal wall musculature and the role of skeletal muscle regeneration and repair is an alternative strategy with the aim to restore the abdominal wall to its pre-pathological mechanical state. Biological scaffolds seeded with muscle progenitor cells have been shown in an animal study that can be used to repair abdominal wall defects with regeneration of skeletal muscle tissue.[204] Alternatively, the potential of biomarkers such as mechano-growth factor (MGF) to predict and potentially treat patients with poor abdominal wall musculature that develop herniation may warrant closer attention despite the negative findings of our study. An animal model may be a useful tool, in the first instance, to establish whether differential gene expression for such markers occurs in the early and late postoperative period. Finally, electrical stimulation is another strategy that may be used to develop a highly differentiated and more functional skeletal muscle in view of the findings of experimental studies showing that neurotisation of engineered skeletal muscle significantly increases force generation compared to non-neurotised constructs.[20 206]

In conclusion, this thesis has critically appraised current evidence on parastomal herniation and presented data on the safety and efficacy of a novel technique for its prevention. It also explored the contribution of the rectus abdominis muscle which may be an important future strategy to prevent herniation.

References

1. Brown H, Randle J. Living with a stoma: a review of the literature. *Journal of clinical nursing* 2005;14(1):74-81.

2. Group SHIAS. High Impact Actions for Stoma Care. http://www.coloplast.co.uk/OstomyCare/Documents/pdfs/High_Impact_Actions_Booklet.pdf, 2010.

3. Pringle W, Swan E. Continuing care after discharge from hospital for stoma patients. *Br J Nurs* 2001;10(19):1275-88.

4. Schafer M. Preventing parastomal hernia with a prosthetic mesh: a five year follow up of a randomised study. *World J Surg* 2009;33(1):122-3.

5. Londono-Schimmer EE, Leong AP, Phillips RK. Life table analysis of stomal complications following colostomy. *Dis Colon Rectum* 1994;37(9):916-20.

6. Dabbas N, Adams K, Pearson K, Royle G. Frequency of abdominal wall hernias: is classical teaching out of date? *JRSM Short Rep* 2011;2(1):5.

7. Bendavid R. The unified theory of hernia formation. *Hernia* 2004;8(3):171-6.

8. Wirtschafter ZT, Bentley JP. Hernias as a Collagen Maturation Defect. *Ann Surg* 1964;160:852-9.

9. Conner WT, Peacock EE, Jr. Some studies on the etiology of inguinal hernia. *Am J Surg* 1973;126(6):732-5.

10. Falco EE, Roth JS, Fisher JP. Skeletal muscle tissue engineering approaches to abdominal wall hernia repair. *Birth Defects Res C Embryo Today* 2008;84(4):315-21.

11. Friedman DW, Boyd CD, Norton P, Greco RS, Boyarsky AH, Mackenzie JW, et al. Increases in type III collagen gene expression and protein synthesis in patients with inguinal hernias. *Ann Surg* 1993;218(6):754-60.

12. Friedman DW, Boyd CD, Mackenzie JW, Norton P, Olson RM, Deak SB. Regulation of collagen gene expression in keloids and hypertrophic scars. *J Surg Res* 1993;55(2):214-22.

13. Wagh PV, Leverich AP, Sun CN, White HJ, Read RC. Direct inguinal herniation in men: a disease of collagen. *J Surg Res* 1974;17(6):425-33.

14. Wagh PV, Read RC. Defective collagen synthesis in inguinal herniation. *Am J Surg* 1972;124(6):819-22.

15. Jorgensen LN, Kallehave F, Christensen E, Siana JE, Gottrup F. Less collagen production in smokers. *Surgery* 1998;123(4):450-5.

16. Witte MB, Thornton FJ, Kiyama T, Efron DT, Schulz GS, Moldawer LL, et al. Metalloproteinase inhibitors and wound healing: a novel enhancer of wound strength. *Surgery* 1998;124(2):464-70.

17. Tarlton JF, Vickery CJ, Leaper DJ, Bailey AJ. Postsurgical wound progression monitored by temporal changes in the expression of matrix metalloproteinase-9. *Br J Dermatol* 1997;137(4):506-16.

18. Ashcroft GS, Horan MA, Herrick SE, Tarnuzzer RW, Schultz GS, Ferguson MW. Age-related differences in the temporal and spatial regulation of matrix metalloproteinases (MMPs) in normal skin and acute cutaneous wounds of healthy humans. *Cell Tissue Res* 1997;290(3):581-91.

19. Yager DR, Zhang LY, Liang HX, Diegelmann RF, Cohen IK. Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *J Invest Dermatol* 1996;107(5):743-8.

20. Zhang L, Li Q, Qin J, Gu Y. Musculature tissue engineering to repair abdominal wall hernia. *Artif Organs* 2012;36(4):348-52.

21. Campion DR. The muscle satellite cell: a review. *Int Rev Cytol* 1984;87:225-51.

22. Hashimoto N, Murase T, Kondo S, Okuda A, Inagawa-Ogashiwa M. Muscle reconstitution by muscle satellite cell descendants with stem cell-like properties. *Development* 2004;131(21):5481-90.

23. DuBay DA, Choi W, Urbanchek MG, Wang X, Adamson B, Dennis RG, et al. Incisional herniation induces decreased abdominal wall compliance via oblique muscle atrophy and fibrosis. *Ann Surg* 2007;245(1):140-6.

24. Amato G, Agrusa A, Romano G, Salamone G, Gulotta G, Silvestri F, et al. Muscle degeneration in inguinal hernia specimens. *Hernia* 2012;16(3):327-31.

25. Amato G, Agrusa A, Romano G, Salamone G, Cocorullo G, Mularo SA, et al. Histological findings in direct inguinal hernia : Investigating the histological changes of the herniated groin looking forward to ascertain the pathogenesis of hernia disease. *Hernia* 2013.

26. Culbertson EJ, Xing L, Wen Y, Franz MG. Reversibility of abdominal wall atrophy and fibrosis after primary or mesh herniorrhaphy. *Ann Surg* 2013;257(1):142-9.

27. Carne PW, Robertson GM, Frizelle FA. Parastomal hernia. *Br J Surg* 2003;90(7):784-93.

28. Carne PW, Frye JN, Robertson GM, Frizelle FA. Parastomal hernia following minimally invasive stoma formation. *ANZ journal of surgery* 2003;73(10):843-5.

29. Goligher J. *Surgery of the Anus, Colon and Rectum.* 5th ed. London: Balliere Tindall, 1984.

30. Park JJ, Del Pino A, Orsay CP, Nelson RL, Pearl RK, Cintron JR, et al. Stoma complications: the Cook County Hospital experience. *Dis Colon Rectum* 1999;42(12):1575-80.

31. Pilgrim CH, McIntyre R, Bailey M. Prospective audit of parastomal hernia: prevalence and associated comorbidities. *Dis Colon Rectum* 2010;53(1):71-6.

32. Shellito PC. Complications of abdominal stoma surgery. *Dis Colon Rectum* 1998;41(12):1562-72.

33. Pearl RK. Parastomal hernias. *World J Surg* 1989;13(5):569-72.

34. Leslie D. The parastomal hernia. *Surg Clin North Am* 1984;64(2):407-15.

35. De Raet J, Delvaux G, Haentjens P, Van Nieuwenhove Y. Waist circumference is an independent risk factor for the development of parastomal hernia after permanent colostomy. *Dis Colon Rectum* 2008;51(12):1806-9.

36. Williams JG, Etherington R, Hayward MW, Hughes LE. Paraileostomy hernia: a clinical and radiological study. *Br J Surg* 1990;77(12):1355-7.

37. Israelsson LA. Preventing and treating parastomal hernia. *World J Surg* 2005;29(8):1086-9.

38. Etherington RJ, Williams JG, Hayward MW, Hughes LE. Demonstration of para-ileostomy herniation using computed tomography. *Clin Radiol* 1990;41(5):333-6.

39. Keeling NJ, Ataullah CM, Wastell C. A survey of glove preferences of general and orthopaedic surgeons in North West Thames Regional Health Authority. *The Journal of hospital infection* 1995;30(4):305-8.

40. Resnick S. New method of bowel stoma formation. *Am J Surg* 1986;152(5):545-8.

41. Koltun L, Benyamin N, Sayfan J. Abdominal stoma fashioned by a used circular stapler. *Dig Surg* 2000;17(2):118-9.

42. Ielpo B, Venditti D, Balassone V, Gioia A, Buonomo O, Petrella G. End-type stapled colostomy in emergency surgery. *Surgical technology international* 2010;20:128-32.

43. Christakis C, Chatzidimitrou C, Kontos N, Papadopoulou S, Karanikas M. Use of intraluminal stapler device for creation of a permanent colostomy. *Tech Coloproctol* 2004;8 Suppl 1:s93-6.

44. Goligher JC. Extraperitoneal colostomy or ileostomy. *Br J Surg* 1958;46(196):97-103.

45. Whittaker M, Goligher JC. A comparison of the results of extraperitoneal and intraperitoneal techniques for construction of terminal iliac colostomies. *Dis Colon Rectum* 1976;19(4):342-4.

46. Marks CG, Ritchie JK. The complications of synchronous combined excision for adenocarcinoma of the rectum at St Mark's Hospital. *Br J Surg* 1975;62(11):901-5.

47. Lian L, Wu XR, He XS, Zou YF, Wu XJ, Lan P, et al. Extraperitoneal vs. intraperitoneal route for permanent colostomy: a meta-analysis of 1,071 patients. *Int J Colorectal Dis* 2012;27(1):59-64.

48. Pearl RK, Prasad ML, Orsay CP, Abcarian H, Tan AB. A survey of technical considerations in the construction of intestinal stomas. *Am Surg* 1985;51(8):462-5.

49. Sjodahl R, Anderberg B, Bolin T. Parastomal hernia in relation to site of the abdominal stoma. *Br J Surg* 1988;75(4):339-41.

50. Ortiz H, Sara MJ, Armendariz P, de Miguel M, Marti J, Chocarro C. Does the frequency of paracolostomy hernias depend on the position of the colostomy in the abdominal wall? *Int J Colorectal Dis* 1994;9(2):65-7.

51. Evans MD, Thomas C, Beaton C, Williams GL, McKain ES, Stephenson BM. Lowering the incidence of stomal herniation: further follow up of the lateral rectus abdominis positioned stoma. *Colorectal Dis.*2011;13(6):716-7.

52. Rozen WM, Ashton MW, Kiil BJ, Grinsell D, Seneviratne S, Corlett RJ, et al. Avoiding denervation of rectus abdominis in DIEP flap harvest II: an intraoperative assessment of the nerves to rectus. *Plastic and reconstructive surgery* 2008;122(5):1321-5.

53. Amato G, Ober E, Romano G, Salamone G, Agrusa A, Gulotta G, et al. Nerve degeneration in inguinal hernia specimens. *Hernia* 2011;15(1):53-8.

54. Wara P, Sorensen K, Berg V. Proximal fecal diversion: review of ten years' experience. *Dis Colon Rectum* 1981;24(2):114-9.

55. Stothert JC, Jr., Brubacher L, Simonowitz DA. Complications of emergency stoma formation. *Arch Surg* 1982;117(3):307-9.

56. Mealy K, O'Broin E, Donohue J, Tanner A, Keane FB. Reversible colostomy--what is the outcome? *Dis Colon Rectum* 1996;39(11):1227-31.

57. Israelsson LA. Parastomal hernias. *Surg Clin North Am* 2008;88(1):113-25, ix.

58. Janes A, Weisby L, Israelsson LA. Parastomal hernia: clinical and radiological definitions. *Hernia* 2011;15(2):189-92.

59. Devlin H, editor. *Operative Surgery, Volume 1*. London: Butterworths, 1983.

60. Moreno-Matias J, Serra-Aracil X, Darnell-Martin A, Bombardo-Junca J, Mora-Lopez L, Alcantara-Moral M, et al. The prevalence of parastomal hernia after formation of an end colostomy. A new clinico-radiological classification. *Colorectal Dis* 2009;11(2):173-7.

61. Nugent KP, Daniels P, Stewart B, Patankar R, Johnson CD. Quality of life in stoma patients. *Dis Colon Rectum* 1999;42(12):1569-74.

62. Makela JT, Niskasaari M. Stoma care problems after stoma surgery in Northern Finland. *Scandinavian journal of surgery : SJS :* 2006;95(1):23-7.

63. Silva MA, Ratnayake G, Deen KI. Quality of life of stoma patients: temporary ileostomy versus colostomy. *World J Surg.* 2003;27(4):421-4.

64. Colquhoun P, Kaiser R, Weiss EG, Efron J, Vernava AM, 3rd, Nogueras JJ, et al. Correlating the Fecal Incontinence Quality-of-Life Score and the SF-36 to a proposed

Ostomy Function Index in patients with a stoma. *Ostomy Wound Manage* 2006;52(12):68-74.

65. Krouse R, Grant M, Ferrell B, Dean G, Nelson R, Chu D. Quality of life outcomes in 599 cancer and non-cancer patients with colostomies. *The Journal of surgical research* 2007;138(1):79-87.

66. Karadag A, Mentes BB, Uner A, Irkorucu O, Ayaz S, Ozkan S. Impact of stomatherapy on quality of life in patients with permanent colostomies or ileostomies. *International journal of colorectal disease* 2003;18(3):234-8.

67. Sprangers MA, Taal BG, Aaronson NK, te Velde A. Quality of life in colorectal cancer. Stoma vs. nonstoma patients. *Dis Colon Rectum* 1995;38(4):361-9.

68. Kald A, Juul KN, Hjortsvang H, Sjodahl RI. Quality of life is impaired in patients with peristomal bulging of a sigmoid colostomy. *Scandinavian journal of gastroenterology* 2008;43(5):627-33.

69. Scarpa M, Ruffolo C, Boetto R, Pozza A, Sadocchi L, Angriman I. Diverting loop ileostomy after restorative proctocolectomy: predictors of poor outcome and poor quality of life. *Colorectal Dis.*2010;12(9):914-20.

70. Thaler K, Dinnewitzer A, Mascha E, Arrigain S, Weiss EG, Nogueras JJ, et al. Long-term outcome and health-related quality of life after laparoscopic and open colectomy for benign disease. *Surgical endoscopy* 2003;17(9):1404-8.

71. Cheatham ML, Safcsak K, Llerena LE, Morrow CE, Jr., Block EF. Long-term physical, mental, and functional consequences of abdominal decompression. *The Journal of trauma* 2004;56(2):237-41; discussion 41-2.

72. Janes A, Cengiz Y, Israelsson LA. Preventing parastomal hernia with a prosthetic mesh: a 5-year follow-up of a randomized study. *World J Surg* 2009;33(1):118-21; discussion 22-3.

73. Nieuwenhuizen J, Kleinrensink GJ, Hop WC, Jeekel J, Lange JF. Indications for incisional hernia repair: an international questionnaire among hernia surgeons. *Hernia* 2008;12(3):223-5.

74. Rubin MS, Schoetz DJ, Jr., Matthews JB. Parastomal hernia. Is stoma relocation superior to fascial repair? *Arch Surg* 1994;129(4):413-8; discussion 18-9.

75. Allen-Mersh TG, Thomson JP. Surgical treatment of colostomy complications. *Br J Surg.* 1988;75(5):416-8.

76. Cheung MT, Chia NH, Chiu WY. Surgical treatment of parastomal hernia complicating sigmoid colostomies. *Dis Colon Rectum m* 2001;44(2):266-70.

77. Guzman-Valdivia G. Incisional hernia at the site of a stoma. *Hernia* 2008;12(5):471-4.

78. Cingi A, Cakir T, Sever A, Aktan AO. Enterostomy site hernias: a clinical and computerized tomographic evaluation. *Dis Colon Rectum* 2006;49(10):1559-63.

79. Luning TH, Spillenaar-Bilgen EJ. Parastomal hernia: complications of extra-peritoneal onlay mesh placement. *Hernia* 2009;13(5):487-90.

80. Guzman-Valdivia G, Guerrero TS, Laurrabaquio HV. Parastomal hernia-repair using mesh and an open technique. *World J Surg* 2008;32(3):465-70.

81. de Ruiter P, Bijnen AB. Ring-reinforced prosthesis for paracolostomy hernia. *Dig Surg* 2005;22(3):152-6.

82. Kanellos I, Vasiliadis K, Angelopoulos S, Kanellos D, Betsis D. Repair of parastomal hernia with the use of polypropylene mesh extraperitoneally. *Tech Coloproctol* 2004;8 Suppl 1:s158-60.

83. Steele SR, Lee P, Martin MJ, Mullenix PS, Sullivan ES. Is parastomal hernia repair with polypropylene mesh safe? *Am J Surg* 2003;185(5):436-40.

84. Geisler DJ, Reilly JC, Vaughan SG, Glennon EJ, Kondylis PD. Safety and outcome of use of nonabsorbable mesh for repair of fascial defects in the presence of open bowel. *Dis Colon Rectum* 2003;46(8):1118-23.

85. Amin SN, Armitage NC, Abercrombie JF, Scholefield JH. Lateral repair of parastomal hernia. *Ann R Coll Surg Engl* 2001;83(3):206-8.

86. Kald A, Landin S, Masreliez C, Sjodahl R. Mesh repair of parastomal hernias: new aspects of the Onlay technique. *Tech Coloproctol* 2001;5(3):169-71.

87. Tekkis PP, Kocher HM, Payne JG. Parastomal hernia repair: modified thorlakson technique, reinforced by polypropylene mesh. *Dis Colon Rectum* 1999;42(11):1505-8.

88. Bayer I, Kyzer S, Chaimoff C. A new approach to primary strengthening of colostomy with Marlex mesh to prevent paracolostomy hernia. *Surg Gynecol Obstet* 1986;163(6):579-80.

89. Abdu RA. Repair of paracolostomy hernias with Marlex mesh. *Dis Colon Rectum* 1982;25(6):529-31.

90. Rosin JD, Bonardi RA. Paracolostomy hernia repair with Marlex mesh: a new technique. *Dis Colon Rectum* 1977;20(4):299-302.

91. Longman RJ, Thomson WH. Mesh repair of parastomal hernias--a safety modification. *Colorectal Dis* 2005;7(3):292-4.

92. Egun A, Hill J, MacLennan I, Pearson RC. Preperitoneal approach to parastomal hernia with coexistent large incisional hernia. *Colorectal Dis* 2002;4(2):132-34.

93. Kasperk R, Klinge U, Schumpelick V. The repair of large parastomal hernias using a midline approach and a prosthetic mesh in the sublay position. *Am J Surg* 2000;179(3):186-8.

94. Ballas KD, Rafailidis SF, Marakis GN, Pavlidis TE, Sakadamis AK. Intraperitoneal ePTFE mesh repair of parastomal hernias. *Hernia* 2006;10(4):350-3.

95. van Sprundel TC, Gerritsen van der Hoop A. Modified technique for parastomal hernia repair in patients with intractable stoma-care problems. *Colorectal Dis* 2005;7(5):445-9.

96. Stelzner S, Hellmich G, Ludwig K. Repair of paracolostomy hernias with a prosthetic mesh in the intraperitoneal onlay position: modified Sugarbaker technique. *Dis Colon Rectum* 2004;47(2):185-91.

97. Morris-Stiff G, Hughes LE. The continuing challenge of parastomal hernia: failure of a novel polypropylene mesh repair. *Ann R Coll Surg Eng* 1998;80(3):184-7.

98. Hofstetter WL, Vukasin P, Ortega AE, Anthone G, Beart RW, Jr. New technique for mesh repair of paracolostomy hernias. *Dis Colon Rectum* 1998;41(8):1054-5.

99. Byers JM, Steinberg JB, Postier RG. Repair of parastomal hernias using polypropylene mesh. *Arch Surg* 1992;127(10):1246-7.

100. Sugarbaker PH. Prosthetic mesh repair of large hernias at the site of colonic stomas. *Surg Gynecol Obstet* 1980;150(4):576-8.

101. Venditti D, Gargiani M, Milito G. Parastomal hernia surgery: personal experience with use of polypropylene mesh. *Tech Coloproctol* 2001;5(2):85-8.

102. Simmermacher RK, van der Lei B, Schakenraad JM, Bleichrodt RP. Improved tissue ingrowth and anchorage of expanded polytetrafluoroethylene by perforation: an experimental study in the rat. *Biomaterials* 1991;12(1):22-4.

103. Koehler RH, Begos D, Berger D, Carey S, LeBlanc K, Park A, et al. Minimal adhesions to ePTFE mesh after laparoscopic ventral incisional hernia repair: reoperative findings in 65 cases. *JSLS : Journal of the Society of Laparoendoscopic Surgeons / Society of Laparoendoscopic Surgeons* 2003;7(4):335-40.

104. Mizrahi H, Bhattacharya P, Parker MC. Laparoscopic slit mesh repair of parastomal hernia using a designated mesh: long-term results. *Surg Endosc* 2012;26(1):267-70.

105. Wara P, Andersen LM. Long-term follow-up of laparoscopic repair of parastomal hernia using a bilayer mesh with a slit. *Surg Endosc* 2010.

106. Hansson BM, de Hingh IH, Bleichrodt RP. Laparoscopic parastomal hernia repair is feasible and safe: early results of a prospective clinical study including 55 consecutive patients. *Surg Endosc* 2007;21(6):989-93.

107. Hansson BM, Bleichrodt RP, de Hingh IH. Laparoscopic parastomal hernia repair using a keyhole technique results in a high recurrence rate. *Surg Endosc* 2009;23(7):1456-9.

108. Pastor DM, Pauli EM, Koltun WA, Haluck RS, Shope TR, Poritz LS. Parastomal hernia repair: a single center experience. *JSLS : Journal of the Society of Laparoendoscopic Surgeons / Society of Laparoendoscopic Surgeons* 2009;13(2):170-5.

109. Muysoms EE, Hauters PJ, Van Nieuwenhove Y, Huten N, Claeys DA. Laparoscopic repair of parastomal hernias: a multi-centre retrospective review and shift in technique. *Acta Chir Belg* 2008;108(4):400-4.

110. Berger D, Bientzle M. Laparoscopic repair of parastomal hernias: a single surgeon's experience in 66 patients. *Dis Colon Rectum. 2*007;50(10):1668-73.

111. Craft RO, Huguet KL, McLemore EC, Harold KL. Laparoscopic parastomal hernia repair. *Hernia* 2008;12(2):137-40.

112. Mancini GJ, McClusky DA, 3rd, Khaitan L, Goldenberg EA, Heniford BT, Novitsky YW, et al. Laparoscopic parastomal hernia repair using a nonslit mesh technique. *Surg Endosc* 2007;21(9):1487-91.

113. LeBlanc KA, Bellanger DE, Whitaker JM, Hausmann MG. Laparoscopic parastomal hernia repair. *Hernia* 2005;9(2):140-4.

114. Safadi B. Laparoscopic repair of parastomal hernias: early results. *Surg Endosc* 2004;18(4):676-80.

115. Kozlowski PM, Wang PC, Winfield HN. Laparoscopic repair of incisional and parastomal hernias after major genitourinary or abdominal surgery. *J Endourol* 2001;15(2):175-9.

116. Voitk A. Simple technique for laparoscopic paracolostomy hernia repair. *Diseases of the colon and rectum* 2000;43(10):1451-3.

117. Zacharakis E, Hettige R, Purkayastha S, Aggarwal R, Athanasiou T, Darzi A, et al. Laparoscopic parastomal hernia repair: a description of the technique and initial results. *Surgical innovation* 2008;15(2):85-9.

118. Brown CN, Finch JG. Which mesh for hernia repair? *Ann R Coll Surg Engl* 2010;92(4):272-8.

119. Carbonell AM, Kercher KW, Sing RF, Heniford BT. Susceptibility of prosthetic biomaterials to infection. *Surg Endosc* 2005;19(12):1670.

120. Schreinemacher MH, Emans PJ, Gijbels MJ, Greve JW, Beets GL, Bouvy ND. Degradation of mesh coatings and intraperitoneal adhesion formation in an experimental model. *Br J Surg* 2009;96(3):305-13.

121. Bohmer RD, Byrne PD, Maddern GJ. A peeling mesh. *Hernia* 2002;6(2):86-7.

122. Helgstrand F, Rosenberg J, Kehlet H, Jorgensen LN, Wara P, Bisgaard T. Risk of morbidity, mortality, and recurrence after parastomal hernia repair: a nationwide study. *Dis Colon Rectum* 2013;56(11):1265-72.

123. Abdel-Baki NA, Bessa SS, Abdel-Razek AH. Comparison of prosthetic mesh repair and tissue repair in the emergency management of incarcerated para-umbilical hernia: a prospective randomized study. *Hernia* 2007;11(2):163-7.

124. Lohsiriwat V, Sridermma W, Akaraviputh T, Boonnuch W, Chinsawangwatthanakol V, Methasate A, et al. Surgical outcomes of Lichtenstein tension-free hernioplasty for acutely incarcerated inguinal hernia. *Surgery today* 2007;37(3):212-4.

125. Nieuwenhuizen J, van Ramshorst GH, ten Brinke JG, de Wit T, van der Harst E, Hop WC, et al. The use of mesh in acute hernia: frequency and outcome in 99 cases. *Hernia :* 2011;15(3):297-300.

126. Wysocki A, Kulawik J, Pozniczek M, Strzalka M. Is the Lichtenstein operation of strangulated groin hernia a safe procedure? *World J Surg* 2006;30(11):2065-70.

127. Campanelli G, Catena F, Ansaloni L. Prosthetic abdominal wall hernia repair in emergency surgery: from polypropylene to biological meshes. *W J Em Surg* 2008;3:33.

128. Gogenur I, Mortensen J, Harvald T, Rosenberg J, Fischer A. Prevention of parastomal hernia by placement of a polypropylene mesh at the primary operation. *Dis Colon Rectum* 2006;49(8):1131-5.

129. Vijayasekar C, Marimuthu K, Jadhav V, Mathew G. Parastomal hernia: Is prevention better than cure? Use of preperitoneal polypropylene mesh at the time of stoma formation. *Tech Coloproctol* 2008;12(4):309-13.

130. Marimuthu K, Vijayasekar C, Ghosh D, Mathew G. Prevention of parastomal hernia using preperitoneal mesh: a prospective observational study. *Colorectal Dis* 2006;8(8):672-5.

131. 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(5):475-81.

132. Serra-Aracil X, Bombardo-Junca J, Moreno-Matias J, Darnell A, Mora-Lopez L, Alcantara-Moral M, et al. Randomized, controlled, prospective trial of the use of a mesh to prevent parastomal hernia. *Annals of surgery* 2009;249(4):583-7.

133. Wijeyekoon SP, Gurusamy K, El-Gendy K, Chan CL. Prevention of parastomal herniation with biologic/composite prosthetic mesh: a systematic review and meta-analysis of randomized controlled trials. *J Am Coll Surg* 2010;211(5):637-45.

134. Godden AR, Daniels IR, Giordano P. The role of biologic meshes in abdominal wall reconstruction. *Colorectal Dis* 2012;14 Suppl 3:7-11.

135. Bellows CF, Alder A, Helton WS. Abdominal wall reconstruction using biological tissue grafts: present status and future opportunities. *Expert Rev Med Devices* 2006;3(5):657-75.

136. Bellon JM, Bujan J, Contreras LA, Carrera-San Martin A, Jurado F. Comparison of a new type of polytetrafluoroethylene patch (Mycro Mesh) and polypropylene prosthesis (Marlex) for repair of abdominal wall defects. *J Am Coll Surg* 1996;183(1):11-8.

137. Cobb WS, Kercher KW, Heniford BT. The argument for lightweight polypropylene mesh in hernia repair. *Surg Innov* 2005;12(1):63-9.

138. Klinge U, Junge K, Spellerberg B, Piroth C, Klosterhalfen B, Schumpelick V. Do multifilament alloplastic meshes increase the infection rate? Analysis of the polymeric surface, the bacteria adherence, and the in vivo consequences in a rat model. *J Biomed Mater Res* 2002;63(6):765-71.

139. Usher FC, Ochsner J, Tuttle LL, Jr. Use of marlex mesh in the repair of incisional hernias. *Am Surg* 1958;24(12):969-74.

140. Usher FC. Hernia Repair with Knitted Polypropylene Mesh. *Surg Gynecol Obstet* 1963;117:239-40.

141. Agarwal BB, Agarwal KA, Mahajan KC. Prospective double-blind randomized controlled study comparing heavy- and lightweight polypropylene mesh in totally extraperitoneal repair of inguinal hernia: early results. *Surg Endosc* 2009;23(2):242-7.

142. Cobb WS, Burns JM, Kercher KW, Matthews BD, James Norton H, Todd Heniford B. Normal intraabdominal pressure in healthy adults. *J Surg Res* 2005;129(2):231-5.

143. Klosterhalfen B, Junge K, Klinge U. The lightweight and large porous mesh concept for hernia repair. *Expert Rev Med Devices* 2005;2(1):103-17.

144. Engelsman AF, van der Mei HC, Ploeg RJ, Busscher HJ. The phenomenon of infection with abdominal wall reconstruction. *Biomaterials* 2007;28(14):2314-27.

145. Simmermacher RK, Schakenraad JM, Bleichrodt RP. Reherniation after repair of the abdominal wall with expanded polytetrafluoroethylene. *J Am Coll Surg* 1994;178(6):613-6.

146. Eriksen JR, Gogenur I, Rosenberg J. Choice of mesh for laparoscopic ventral hernia repair. *Hernia* 2007;11(6):481-92.

147. Diaz JJ, Jr., Gray BW, Dobson JM, Grogan EL, May AK, Miller R, et al. Repair of giant abdominal hernias: does the type of prosthesis matter? *Am Surg* 2004;70(5):396-401; discussion 01-2.

148. McGinty JJ, Hogle NJ, McCarthy H, Fowler DL. A comparative study of adhesion formation and abdominal wall ingrowth after laparoscopic ventral hernia repair in a porcine model using multiple types of mesh. *Surg Endosc* 2005;19(6):786-90.

149. Bachman S, Ramshaw B. Prosthetic material in ventral hernia repair: how do I choose? *Surg Clin North Am* 2008;88(1):101-12, ix.

150. Park TG. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* 1995;16(15):1123-30.

151. Craig PH, Williams JA, Davis KW, Magoun AD, Levy AJ, Bogdansky S, et al. A biologic comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures. *Surg Gynecol Obstet* 1975;141(1):1-10.

152. Klinge U, Schumpelick V, Klosterhalfen B. Functional assessment and tissue response of short- and long-term absorbable surgical meshes. *Biomaterials* 2001;22(11):1415-24.

153. Courtney CA, Duffy K, Serpell MG, O'Dwyer PJ. Outcome of patients with severe chronic pain following repair of groin hernia. *Br J Surg* 2002;89(10):1310-4.

154. Pans A, Elen P, Dewe W, Desaive C. Long-term results of polyglactin mesh for the prevention of incisional hernias in obese patients. *World J Surg* 1998;22(5):479-82; discussion 82-3.

155. Tyrell J, Silberman H, Chandrasoma P, Niland J, Shull J. Absorbable versus permanent mesh in abdominal operations. *Surg Gynecol Obstet* 1989;168(3):227-32.

156. Smart NJ, Bryan N, Hunt JA. A scientific evidence for the efficacy of biologic implants for soft tissue reconstruction. *Colorectal Dis* 2012;14 Suppl 3:1-6.

157. Hubner M, Streit D, Hahnloser D. Biological materials in colorectal surgery: current applications and potential for the future. *Colorectal Dis* 2012;14 Suppl 3:34-9.

158. Slater NJ, Hansson BM, Buyne OR, Hendriks T, Bleichrodt RP. Repair of parastomal hernias with biologic grafts: a systematic review. *J Gastrointest Surg* 2011;15(7):1252-8.

159. Holl-Allen RT. Porcine dermal collagen implants in man. *J R Coll Surg Edinb* 1984;29(3):151-3.

160. James NL, Poole-Warren LA, Schindhelm K, Milthorpe BK, Mitchell RM, Mitchell RE, et al. Comparative evaluation of treated bovine pericardium as a xenograft for hernia repair. *Biomaterials* 1991;12(9):801-9.

161. van der Laan JS, Lopez GP, van Wachem PB, Nieuwenhuis P, Ratner BD, Bleichrodt RP, et al. TFE-plasma polymerized dermal sheep collagen for the repair of abdominal wall defects. *Int J Artif Organs* 1991;14(10):661-6.

162. Oliver RF, Hulme MJ, Mudie A, Grant RA. Skin collagen allografts in the rat. *Nature* 1975;258(5535):537-9.

163. van Wachem PB, van Luyn MJ, Olde Damink LH, Dijkstra PJ, Feijen J, Nieuwenhuis P. Tissue regenerating capacity of carbodiimide-crosslinked dermal sheep collagen during repair of the abdominal wall. *Int J Artif Organs* 1994;17(4):230-9.

164. Inan I, Gervaz P, Hagen M, Morel P. Multimedia article. Laparoscopic repair of parastomal hernia using a porcine dermal collagen (Permacol) implant. *Dis Colon Rectum* 2007;50(9):1465.

165. Mitchell CR, Cima RR. A Novel Technique for the Repair of Urostomal Hernias Using Human Acellular Dermal Matrix. *Urology* 2010.

166. Franklin ME, Jr., Trevino JM, Portillo G, Vela I, Glass JL, Gonzalez JJ. The use of porcine small intestinal submucosa as a prosthetic material for laparoscopic hernia repair in infected and potentially contaminated fields: long-term follow-up. *Surg Endosc* 2008;22(9):1941-6.

167. Melman L, Jenkins ED, Hamilton NA, Bender LC, Brodt MD, Deeken CR, et al. Early biocompatibility of crosslinked and non-crosslinked biologic meshes in a porcine model of ventral hernia repair. *Hernia* 2011;15(2):157-64.

168. Mulier KE, Nguyen AH, Delaney JP, Marquez S. Comparison of Permacol and Strattice for the repair of abdominal wall defects. *Hernia* 2011;15(3):315-9.

169. Loganathan A, Ainslie WG, Wedgwood KR. Initial evaluation of Permacol bioprosthesis for the repair of complex incisional and parastomal hernias. *Surgeon* 2010;8(4):202-5.

170. Hiles M, Record Ritchie RD, Altizer AM. Are biologic grafts effective for hernia repair?: a systematic review of the literature. *Surg Innov* 2009;16(1):26-37.

171. Ghahremani GG, Jimenez MA, Rosenfeld M, Rochester D. CT diagnosis of occult incisional hernias. *AJR. American journal of roentgenology* 1987;148(1):139-42.

172. Williams NS, Nair R, Bhan C. Stapled mesh stoma reinforcement technique (SMART)--a procedure to prevent parastomal herniation. *Ann R Coll Surg Eng* 2011;93(2):169.

173. Hotouras A, Murphy J, Power N, Williams NS, Chan CL. Radiological incidence of parastomal herniation in cancer patients with permanent colostomy: what is the ideal size of the surgical aperture? *Int J Surg* 2013;11(5):425-7.

174. Hotouras A, Murphy J, Thaha M, Chan C. The persistent challenge of parastomal herniation: A review of the literature and future developments. *Colorectal Dis* 2013.

175. Goldspink G, Harridge SD. Growth factors and muscle ageing. *Exp Gerontol* 2004;39(10):1433-8.

176. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SD. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* 2003;547(Pt 1):247-54.

177. Goldspink G, Scutt A, Loughna PT, Wells DJ, Jaenicke T, Gerlach GF. Gene expression in skeletal muscle in response to stretch and force generation. *Am J Physiol* 1992;262(3 Pt 2):R356-63.

178. Goldspink G. Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *J Anat* 1999;194 (Pt 3):323-34.

179. Yang S, Alnaqeeb M, Simpson H, Goldspink G. Cloning and characterization of an IGF-1 isoform expressed in skeletal muscle subjected to stretch. *J Muscle Res Cell Motil* 1996;17(4):487-95.

180. Goldspink G. Mechanical signals, IGF-I gene splicing, and muscle adaptation. *Physiology (Bethesda)* 2005;20:232-8.

181. Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proc Natl Acad Sci U S A* 1998;95(26):15603-7.

182. Yang SY, Goldspink G. Different roles of the IGF-I Ec peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. *FEBS Lett* 2002;522(1-3):156-60.

183. Hill M, Goldspink G. Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. *J Physiol* 2003;549(Pt 2):409-18.

184. Haddad F, Adams GR. Selected contribution: acute cellular and molecular responses to resistance exercise. *J Appl Physiol* 2002;93(1):394-403.

185. Brooks SV, Faulkner JA. Contraction-induced injury: recovery of skeletal muscles in young and old mice. *Am J Physiol* 1990;258(3 Pt 1):C436-42.

186. Renault V, Thornell LE, Eriksson PO, Butler-Browne G, Mouly V. Regenerative potential of human skeletal muscle during aging. *Aging Cell* 2002;1(2):132-9.

187. Owino V, Yang SY, Goldspink G. Age-related loss of skeletal muscle function and the inability to express the autocrine form of insulin-like growth factor-1 (MGF) in response to mechanical overload. *FEBS Lett* 2001;505(2):259-63.

188. Hotouras A, Murphy J, Thaha M, Chan CL. The persistent challenge of parastomal herniation: a review of the literature and future developments. *Colorectal Dis* 2013;15(5):e202-14.

189. Stephenson BM, Evans MD, Hilton J, McKain ES, Williams GL. Minimal anatomical disruption in stoma formation: the lateral rectus abdominis positioned stoma (LRAPS). *Colorectal Dis* 2010;12(10):1049-52.

190. Cortes E, te Fong LF, Hameed M, Harridge S, Maclean A, Yang SY, et al. Insulin-like growth factor-1 gene splice variants as markers of muscle damage in levator ani muscle after the first vaginal delivery. *Am J Obstet Gynecol* 2005;193(1):64-70.

191. Dubay DA, Franz MG. Acute wound healing: the biology of acute wound failure. *Surg Clin North Am* 2003;83(3):463-81.

192. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *J Appl Physiol* 1998;85(1):115-22.

193. Kuk JL, Church TS, Blair SN, Ross R. Associations between changes in abdominal and thigh muscle quantity and quality. *Med Sci Sports Exerc* 2008;40(7):1277-81.

194. Lee JS, He K, Harbaugh CM, Schaubel DE, Sonnenday CJ, Wang SC, et al. Frailty, core muscle size, and mortality in patients undergoing open abdominal aortic aneurysm repair. *J Vasc Surg* 2011;53(4):912-7.

195. Strasser EM, Draskovits T, Praschak M, Quittan M, Graf A. Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age (Dordr)* 2013.

196. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol (1985)* 2000;89(1):104-10.

197. Nordal HJ, Dietrichson P, Eldevik P, Gronseth K. Fat infiltration, atrophy and hypertrophy of skeletal muscles demonstrated by X-ray computed tomography in neurological patients. *Acta Neurol Scand* 1988;77(2):115-22.

198. Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* 1999;48(4):839-47.

199. Goodman P, Balachandran S, Guinto FC, Jr. Postoperative atrophy of posterolateral chest wall musculature: CT demonstration. *J Comput Assist Tomogr* 1993;17(1):63-6.

200. Dorado C, Calbet JA, Lopez-Gordillo A, Alayon S, Sanchis-Moysi J. Marked effects of Pilates on the abdominal muscles: a longitudinal magnetic resonance imaging study. *Med Sci Sports Exerc* 2012;44(8):1589-94.

201. Burden AM, C GR. Abdominal and hip flexor muscle activity during 2 minutes of sit-ups and curl-ups. *J Strength Cond Res* 2013;27(8):2119-28.

202. Englesbe MJ, Lee JS, He K, Fan L, Schaubel DE, Sheetz KH, et al. Analytic morphomics, core muscle size, and surgical outcomes. *Ann Surg* 2012;256(2):255-61.

203. Conconi MT, De Coppi P, Bellini S, Zara G, Sabatti M, Marzaro M, et al. Homologous muscle acellular matrix seeded with autologous myoblasts as a tissue-engineering approach to abdominal wall-defect repair. *Biomaterials* 2005;26(15):2567-74.

204. Ayele T, Zuki AB, Noorjahan BM, Noordin MM. Tissue engineering approach to repair abdominal wall defects using cell-seeded bovine tunica vaginalis in a rabbit model. *J Mater Sci Mater Med* 2010;21(5):1721-30.

205. Mylonakis E, Scarpa M, Barollo M, Yarnoz C, Keighley MR. Life table analysis of hernia following end colostomy construction. *Colorectal disease* 2001;3(5):334-7.

206. Dennis RG, Kosnik PE, 2nd, Gilbert ME, Faulkner JA. Excitability and contractility of skeletal muscle engineered from primary cultures and cell lines. *Am J Physiol Cell Physiol* 2001;280(2):C288-95.

Appendix 1

EuroQol EQ-5D Health Survey

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about	❑
I have some problems in walking about	❑
I am confined to bed	❑

Self-Care

I have no problems with self-care	❑
I have some problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

Usual Activities *(e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities	❑
I have some problems with performing my usual activities	❑
I am unable to perform my usual activities	❑

Pain/Discomfort

I have no pain or discomfort	❑
I have moderate pain or discomfort	❑
I have extreme pain or discomfort	❑

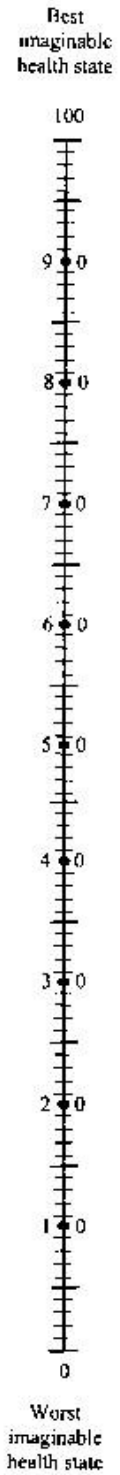
Anxiety/Depression

I am not anxious or depressed	❑
I am moderately anxious or depressed	❑
I am extremely anxious or depressed	❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



3

Appendix II

RNA isolation from human muscle tissue

This section gives details for disrupting and homogenizing stabilized tissues for RNA purification purposes.

1. Add 30-50mg of tissue to 200ul TRIZOL in a 2ml round bottomed RNAse-free tube. Add 2 x 5mm stainless steel beads and place in tissue lyser (with balance) and shake for 2 minutes at 25Hz.
2. Remove beads and add 800ul TRIZOL.
3. Add 200µl 2-bromo-3-chloropropanol (chloroform, Sigma-Aldrich) (per ml TRIZOL) to the homogenised tissue and shake for 15s.
4. Transfer the homogenate (1ml aliquots) into an appropriate number of pre-spun (13,000 rpm for 30s) phase lock gel 2ml tubes Incubate at room temperature for 3 minutes.
5. Centrifuge (using centrifuge in the cold room) at 11,800rpm for 15 minutes.
6. Remove clear aqueous upper phase into a new RNAse-free eppendorf tube.
7. Add 500ul 2-propanol (Sigma-Aldrich), invert to mix and incubate at room temperature for 10minutes.
8. Centrifuge (using centrifuge in the cold room) at 11,800rpm for 10 minutes.
9. Discard supernatant carefully as not to disrupt or remove pellet. The pellet is sometimes difficult to see and appears as a smear on the side of the tube.
10. Wash pellet with 1ml 75% Ethanol (Sigma-Aldrich)/H2O and vortex.

11. Centrifuge at 9,200 rpm for 5 minutes
12. Remove supernatant and air dry for 5minutes. Add 10ul RNAase-free H_2O and quantify using NanoDrop®.
13. Record RNA concentration on the tube and on the record sheet.

Appendix III

Reverse transcription protocol using OmniScript Reverse Transcriptase Kit (Qiagen, UK)

The QIAGEN reverse transcriptase kit contains Omniscript reverse transcriptase, x10 buffer RT, dNTP (5mM) and RNA free water. RNase inhibitor (Recombinant RNasin Ribonuclease inhibitor (Promega-N2511) and oligo-dT primers (oligo dT15 primer (Promega-C1101) were supplied separately. The components are mixed to create a 20µl solution as listed in the table below:

Component	Volume/reaction	Final concentration
X10 Buffer RT	2µl	X1
dNTP (5mM)	2µl	0.5mM each dNTP
Oligo dT_{15} (10µM)	2µl	1µM
RNasin inhibitor (40u/µl)	0.1µl	4 units
Omniscript RT	1µl	4 units
Master Mix volume	7.10µl	
RNA Template	Variable*	
RNase-free water	Variable*	
Sample volume	12.9ul	
Total reaction volume	**20µl**	

Procedure

1. Thaw the RNA template, the primer solutions, 10x Buffer RT, dNTP Mix and RNase-free water at room temperature (15–25°C). Store on ice immediately after thawing. Mix each solution by vortexing and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Commercially available RNase inhibitor is commonly supplied at 40 units/μl. Dilute the RNase inhibitor to a final concentration of 10 units/μl in ice-cold 1x Buffer RT (dilute an aliquot of 10x Buffer RT accordingly using the RNase-free water supplied). Mix carefully by vortexing for no more than 5 seconds, and centrifuge briefly to collect residual liquid from the sides of the tube.
3. Prepare a fresh master mix on ice according to the table above. Mix thoroughly and carefully by vortexing for no more than 5 seconds. Centrifuge briefly to collect residual liquid from the walls of the tube, and store on ice.
4. Once RNA has been extracted and quantified, determine the volume of sample in μl required for 1μg of total RNA. Add template RNA to the individual tubes containing the master mix. Mix thoroughly and carefully by vortexing for no more than 5 seconds. Centrifuge briefly to collect residual liquid from the walls of the tubes.
5. Incubate for 60 min at 37°C.
6. Store reverse- transcription reactions on ice and proceed directly with PCR, or for long-term storage, store reverse- transcription reactions at – 20°C.

Appendix IV

Generation of qPCR standards

Component	Volume/reaction	Final concentration
HotStarTaq Master Mix	12.5μl	2.5u HST DNA polymerase 1.5mM $MgCl_2$ 200μM each dNTP x1 PCR buffer
Forward primer (10uM stock)	0.75μl	300nM
Reverse primer (10uM stock)	0.75μl	300nM
Distilled	7.5μl	
Master Mix volume	21.5μl	
cDNA template (100ng)	3.5μl	
Total reaction volume	25μl	

When using HotStarTaq Master Mix, each PCR program must start with an initial heat activation step at 95°C for 15 min. The reactions conditions are as follows:

Initial activation step 15 min @ 95°C

Denaturation 1 min @ 94°C

Annealing 1 min @ 60°C

Extension 2 min @ 72°C

Number of cycles ~ 35

Final extension 10 min @ 72°C and hold at 4°C

Appendix V

Gel extraction and purification of RT-PCR product

1. Excise DNA fragment from the agarose gel with a clean sharp scalpel.

2. Weigh the gel slice in a colourless tube. Add 3 volumes of buffer QG to 1 volume of the gel e.g. 300ul buffer to 100mg of gel.

3. Incubate at 50oC for 10 min (or until the slice has completely dissolved). Mix by vortexing the tube every 2-3 minutes.

4. Check that the colour of the mixture is still yellow. If the colour is orange or violet, add 10ul of 3M sodium acetate.

5. Add 1 gel volume of isopropanol to the sample and mix. e.g. if gel sample was 100mg add 100ul.

6. Place QIAquick spin column in a 2ml collection tube. Apply 800ul sample mix to column and centrifuge for 1 minute @ 13,000rpm. Discard flow-through. For any remaining sample, load and spin again.

7. Add 500ul of buffer QG to column and centrifuge for 1 minute at 13,000rpm.

8. Add 750ul of buffer PE to column and centrifuge for 1 minute @ 13,00rpm.

9. Discard flow-through and centrifuge for 1 minute @ 13,000rpm.

10. Place QIAquick column in a 1.5ml micro centrifuge tube. Add 30ul buffer EB to the centre of the membrane and centrifuge for 1 minute@13, 000rpm. Quantify DNA using the NanoDrop.

Appendix VI

Calculation of gene copy number

Firstly, the molecular weight (MW) of a PCR gene product of size **A** bp must be calculated.

The average MW of a dNTP (A, T, G, C) = 330 Da.

As the cDNA product is double stranded, the dNTP MW = **660** Da. (This calculation is based on the assumption that there is an equal number of all bases in the product).

The MW of PCR product **B** = average dNTP MW x A bp of PCR product.

B = **660** x **A**

Now the number of copies of PCR product in 1ng is calculated:

Avocadro's constant = 6.02×10^{23} molecules in 1 mole and is equal to molecular weight in grams.

B grams of gene product = 6.02×10^{23} molecules.

1ng = 10^{-9} g

Therefore, in 1ng of gene product there is = 6.02x*1023***B**×*10-9*= **C** copies.

Lastly, the number of copies of PCR product in 1 µl is calculated using the concentration recorded from the Nanodrop-1000 spectrophotometer:

There is **C** copies in 1ng of gene product.

The concentration of cDNA in a sample is **D** ng/µl

Therefore, number of copies in 1ng x cDNA concentration = **E** copies/µl

C x **D** = **E**