The development of a polymer patch for the treatment of oesophageal leaks and perforations

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‘I, Nicholas Newton, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

Abstract

Oesophageal leaks and perforations carry mortality and morbidity. Management options include surgery, stents, drains, and negative pressure therapy; all current treatment options, however, have significant associated morbidity. Here we investigate an alternative approach using a temporary polymer patch to adhere to and seal the oesophageal defect whilst supporting healing and function without damaging local tissue. This approach could offer a timely, cost effective and minimally invasive approach, especially for environments where specialised and complex surgery may not be available such as resource limited military and humanitarian healthcare settings. To quantitatively compare perforation management options (including the novel patch) a novel *ex vivo* model of oesophageal perforation repair was developed. The patch had a degradable poly(ε-caprolactone urea) urethane backbone with a polyhedral oligomeric silsesquioxane (POSS PCLU) component.

The study successfully developed an ex vivo porcine oesophagus bench top model for testing the strength of oesophageal repair techniques. The model allowed a maximum pressure of 100kPa and was capable of quantitatively assessing perforation repair approaches. A major consideration in the patch approach to perforation repair is the adhesion of the patch to the oesophagus. The study demonstrates that the surface chemistry of the patch can be modified to improve adhesion and increase attachment strength. Here we found that both modifications of the tissue adhesion site in vivo (by removal of the outer epithelial layer of the oesophagus) and by modifying the surface chemistry of the patch (by plasma treatment) it was possible to enhance fibrin patch adhesion to the oesophagus. The optimisation of the patch surface chemistry and host tissue site for fibrin-based adhesion could have an impact in the medical use of polymer patches throughout the body.

Impact Statement

This project has looked at the development of a bench top model of oesophageal perforation to test an adhesive polymer patch for treatment of oesophageal leaks and perforations. This is the first time a patch, using a biopolymer and biological adhesive, has been assessed for oesophagus repair, with the intention of offering a cost-effective, low tech and minimally invasive solution to small perforation management. Further translation of the patch could develop a relatively low risk, low cost and simple alternative for oesophagus repair.

In developing the patch approach, an ex-vivo model to test oesophagus perforation repair was developed. This simple bench-top model is a cost effective and quantitative alternative to expensive in vivo models for assessing oesophagus perforation management and could be utilised for other hollow organs. It is also in line with the 3Rs and would reduce the use of live animal models.

For the first time differences in fibrin glue adhesion were demonstrated between epithelium and underlying muscle in the oesophagus. This may impact clinical application of fibrin adhesive patches and clinical approach. Furthermore, it was found that modification of the polymer surface can enhanced fibrin glue adhesion. This could have important consequences for the development and optimisation of a range of biomaterials used with fibrin adhesives.

This project, based around laboratory techniques and bench-top models, lays the ground work for animal studies leading to human trials of a clinical product that would allow a minimally invasive single treatment for oesophageal injuries that would be highly beneficial in a clinical setting. This would be a clinical product that would have wide application, reducing the morbidity associated with oesophageal injuries and the subsequent healthcare logistical and financial burden.

Acknowledgements

Coming from a clinical surgical background one of my motivations for engaging in this project was to broaden my understanding and knowledge of basic science across a number of disciplines. I am incredibly grateful for the support of my supervisor Gavin Jell who has supported me at every stage of this research and subsequent writing. A number of other people supported me during this project from the PhD students and lab technicians in the UCL Division of Surgery & Interventional Science and UCL Mechanical Engineering including Benyamin Rahmani, Stephanie Bogan, Deepak Kalaskar and many others without whom I could not have completed this work. I am also very grateful for the financial and academic support of the Defence Medical Services Academic Department of Military Surgery and Trauma in particular Surgeon Captain Rory Rickard, Professor of Military Surgery.

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1 Introduction

1.1 What are the clinical problems associated with oesophageal leaks and perforations?

Oesophageal perforation is an uncommon but serious condition associated with significant mortality and morbidity. Mortality can vary between 5-30%[1] depending on underlying cause and clinical circumstances. A delay in diagnosis and treatment beyond 24 hours can increase mortality to over 50%. Many factors affect the outcome from oesophageal perforations including mechanism of injury, patient factors and treatment modality giving a wide variation in mortality and morbidity [2-4]. The key to successful management of oesophageal injuries is to control contamination, treat infection and maintain nutrition throughout the healing process. Current treatments include open surgery, endoscopic placement of stents, clips and drains and feeding regimes that bypass the injury are also used. These carry morbidity in their own right and require extended periods of time in hospital[5].

1.2 The Oesophagus

1.2.1 Anatomy

The oesophagus is a muscular tube running from the oropharynx to the stomach. It is broadly divided into a cervical section, a thoracic section and an abdominal section (Figure 1) and for most of its course lies within the posterior mediastinum.

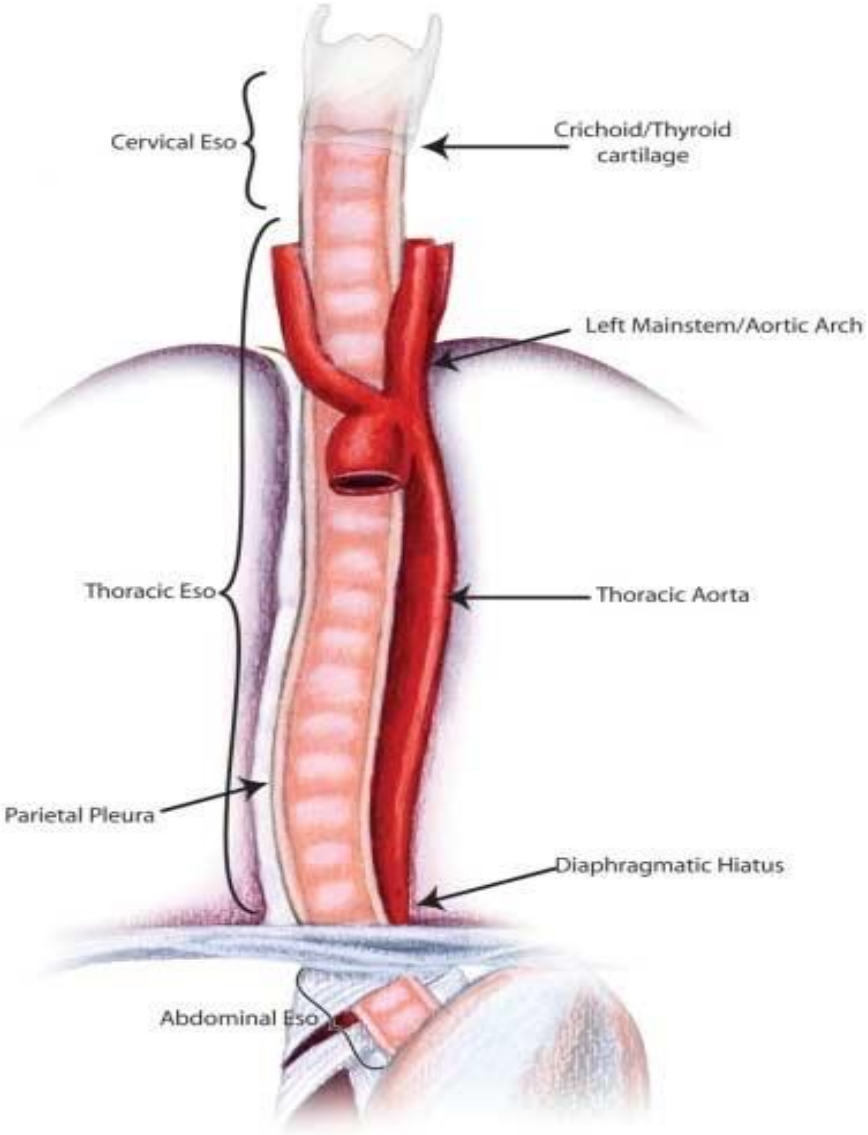


Figure 1.1: Gross anatomy of the oesophagus, demonstrating the relationship of the three sections of the oesophagus to the diaphragm, the aorta and the cricoid cartilage. From Plott et al. 2007[6].

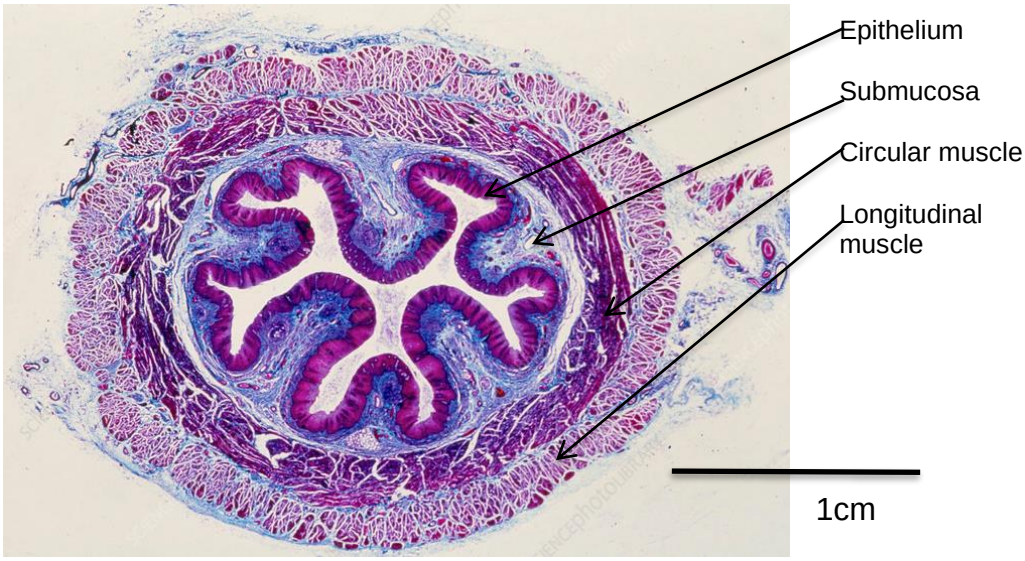


Figure 1.2: Cross sectional light microscopy image showing epithelial lining, submucosa and muscle layers. https://www.sciencephoto.com/contributor/bio/©

The wall of the oesophagus is composed of smooth muscle and striated muscle and has sphincter mechanisms at the top (the upper oesophageal sphincter) and the bottom (the lower oesophageal sphincter). In the proximal third of the oesophagus the muscle is predominantly striated in the distal third is predominantly smooth and in the middle third there is a transition between the two types.

1.2.2 Physiology

The oesophagus allows the transit of food boluses from the oropharynx to the stomach while preventing reflux of gastric contents. The upper and lower oesophageal sphincters contribute to controlling the transit of food boluses down the oesophagus while controlling reflux of gastric contents. Peristalsis is initiated by the swallowing reflex and passes the bolus of food down the oesophagus. Normal peristaltic wave pressures are 4-16kPa while the lower oesophageal sphincter has a resting pressure of 1.3-2kPa preventing reflux gastric contents[7].

1.3 Leaks and Perforations of the Oesophagus

1.3.1 Clinical Presentation

Oesophageal perforations can be difficult to diagnose due to the presenting symptoms being similar to other serious medical conditions such as

myocardial infarction or pneumonia (shortness of breath, chest pain, fever); if left untreated oesophageal perforations can be life threatening with mortality of up to 100% reported[7]. Typical symptoms of perforations include chest pain, dysphagia, fever and surgical emphysema[8, 9].

In the context of traumatic perforations coexisting injuries may take precedence in the initial management with the oesophageal injury only becoming apparent when sepsis within the mediastinum becomes established [4, 10]. Morbidity associated with oesophageal perforations is due to spillage of contents of the gastrointestinal tract into the sterile space of the mediastinum. Severe sepsis with associated multi-organ failure can set in rapidly [4, 11]. Mortality and morbidity associated with oesophageal perforations increases markedly if there is a delay in management although the ideal timing of intervention is not known [4]. A number of studies have looked at delay in diagnosis, treatment and outcome and have identified adverse outcomes associated with delays between 12-48 hours [3, 4, 12, 13]. These studies had heterogeneous patient populations and were not prospective or randomized, making it difficult to draw clear conclusions.

1.3.2 Mechanisms of injury and epidemiology

There are a number of recognised mechanisms that can result in perforation of the oesophagus with variable incidence as summarised in table 1.The commonest cause of injury in the literature is iatrogenic[14] accounting for 59% of cases in a series of 559 patients, while in the same series spontaneous perforations accounted for 15% of cases, foreign bodies 12% and traumatic injuries 9%. Overall incidence of oesophageal perforation due to trauma is estimated to be less than 0.2%[7]. Oesophageal injuries are often associated with other injuries, up to 88% in a study by Asensio et al.[15]. This study, conducted on behalf of the American Association for the Surgery of Trauma was a multicentre review of oesophageal injuries. The overall mortality in the cohort of 405 patients was 19% however intrathoracic oesophageal injuries had a mortality of 55%.

There are many different treatment options available and while open surgery may be appropriate for the most serious cases less invasive techniques also exist including endoscopic and minimally invasive techniques using stents and negative pressure therapy, [16-18].

1.3.3 Iatrogenic Perforations

Endoscopic procedures of the upper gastrointestinal tract account for the majority of oesophageal perforations [14, 19]. While simple diagnostic endoscopy has a low rate of perforation typically quoted as 0.03%, therapeutic procedures carry a much greater risk that is often difficult to quantify due to the wide variety of interventions carried out.

The incidence of distal oesophageal perforation due to balloon dilatation of achalasia ranges from 2-6% and increases after repeated procedures[20] although Hagel et al. reported a perforation rate of just 0.53%[21], and Lakhdar-Idrissi et al. reported a perforation rate of 0.8%[22]. Injection sclerotherapy for the treatment of oesophageal varices can cause full thickness necrosis resulting in perforation which occurs in 1-3% of patients[23].

Endoscopic resection of cancers is an increasingly used technique as the incidence of oesophageal cancer rises and screening programs detect abnormalities earlier[24]. Endoscopic resection allows superficial cancers to be removed without the requirement for open surgery and the associated mortality and morbidity. However, endoscopic resection techniques carry a significant risk of perforation that is dependent on the level the resection is taking place and the level of experience of the operator. A perforation rate of 5% was found in a 2014 systematic review and meta-analysis looking at 15 studies involving 776 patients undergoing endoscopic submucosal dissection for oesophageal lesions [25]. Another systematic review also published in 2014 found 21 studies for inclusion with 1152 patients and a perforation rate of 1% [26]. Van Vilsteren et al.[27] reviewed the results of 6 endoscopists performing their first 20 endoscopic oesophageal resections using a variety of techniques performed within the setting of an intensive, comprehensive training program.

The perforation rate was 5% although the majority of these were recognized immediately and were associated with no significant morbidity.

The oesophagus may also be damaged during laparoscopic surgery around the diaphragmatic hiatus for conditions such hiatus hernia repair, fundoplication and myotomy [28].

1.3.4 External Trauma

Oesophageal trauma is an uncommon but devastating injury. Penetrating trauma is much more common than blunt trauma with only a small number of cases of blunt oesophageal trauma reported in the medical literature [29-32]. Barotrauma is documented in a handful of case reports detailing injury to the oesophagus due to high pressure gas and these are treated in a similar manner to penetrating oesophageal injuries [33-37].

Blast injury represents a specific subset of traumatic injury that differs from penetrating and blunt mechanisms. Blast injury patterns are particularly important in the setting of military trauma as well as in the management of injuries caused by terrorist action. Blast injury to the oesophagus may be due to the pressure wave (primary blast injury) causing barotrauma [10], penetrating injury from fragments (tertiary blast injury) [38, 39] or burns to the oesophagus (quaternary blast injury) [40]. Other suggested mechanisms of oesophageal injury following both blast and blunt trauma is localized ischaemia, caused by shearing of the small blood vessels supplying the oesophagus from the aorta [31, 41].

Penetrating oesophageal trauma most commonly affects the cervical oesophagus where it is relatively unprotected by the bony structures of the thorax. The cervical oesophagus is located deep in the neck and is often associated with injuries to the trachea, great vessels or spinal cord. It is these injuries that account for much of the observed morbidity[38, 42].

Asensio et al on behalf of the American Association for the Surgery of Trauma performed a retrospective review of penetrating oesophageal injuries

from 34 institutions over a 10-year period[15], 405 patients were identified and gunshot wounds accounted for three quarters of the injuries. Overall mortality was 19%, the majority of these deaths, however, occurred early in the treatment pathway either in the Emergency Department or during the initial surgery due to associated injuries. 38% of survivors experienced complications of which half were considered due to the oesophageal injury giving an overall oesophageal related complication rate of 31%. The authors noted that while a delay in getting to theatre due to preoperative investigations did not affect mortality it did seem to increase infection related complications including abscess, mediastinitis and empyema.

In addition to the above studies three large epidemiological studies have looked at oesophageal injuries. A National Trauma Data Bank study from 2011 identified 227 patients from 107 level 1 and level 2 trauma centres over a 2-year period with penetrating injuries to the oesophagus [43], Makhani et al.[44] performed a retrospective study looking at the Pennsylvania Trauma Outcome Study database identifying 327 patients with oesophageal injury over a 7-year period and Sheely et al.[42] looked at 700 cases of penetrating neck trauma over a 22-year period. This last study identified 39 (5.5%) patients with oesophageal injury. The most common associated injury was to the trachea however any structure in the neck is at risk[42]. Mortality was 44% with 92% of these occurring within 24 hours of admission to hospital in the National Database. In the Pennsylvania study gunshot wounds or stabbings were the most common mechanism; 35.8% of patients required surgical intervention and the overall mortality was 20.5%.

The thoracic oesophagus is much better protected as it located deep within the mediastinum and is small compared to surrounding structures. Cornwell reported an incidence of 0.7% oesophageal injury in a series of 1 961 gunshot wounds to the chest [45]. A retrospective review of war casualties admitted to Split University Hospital during the 1991-1995 conflicts in Croatia identified 5 patients with oesophageal injuries accounting for 0.2% of the total. All patients were managed operatively and one died. This centre was seeing patients transferred from other facilities and it is likely that the true

incidence and mortality was greater as many patients would not have survived the initial injury and treatment [46].

Breeze et al in 2012 described a case series of cervical injuries seen in UK service personnel from 2004 to 2008. The overall mortality was 63%, 15% had injuries to the pharynx or cervical oesophagus [38]. Mortality was predominantly due to vascular and neurological structures being injured.

1.3.5 Boerhaave's Syndrome

Hermann Boerhaave, a Dutch physician, described a case of spontaneous oesophageal perforation after performing the autopsy of Baron von Wassenaer, Grand Admiral of the Dutch Navy in 1724. The previously fit and well Admiral developed severe chest pain following forced vomiting after indulging a large quantity of food and wine [47].

Spontaneous perforation of the oesophagus (Boerhaave's Syndrome) is rare, accounting for only 15% of all oesophageal perforations, but can present a diagnostic challenge as the presenting symptoms mimic other life threatening conditions including myocardial infarction, pneumonia and pulmonary embolism resulting in delays that adversely affect outcome by delaying definitive diagnosis and treatment[48]. Boerhaave's Syndrome typically presents with severe pain following vomiting, there may be associated dyspnoea and surgical emphysema[5]. Mackler's triad of vomiting, pain and surgical emphysema is considered pathognomonic but is only present in 5-50% of cases[4, 5, 49, 50].

The likely mechanism of injury is a sudden rise in intraluminal pressure through a patent lower oesophageal sphincter against a constricted cricopharyngeal muscle[48, 51] resulting in a full thickness oesophageal rupture. The perforation of Boerhaave's is typically in the lower third of the oesophagus on the left posterolateral aspect where the longitudinal muscle fibres start to thin as at the level of gastro-oesophageal junction[3, 51].

As has been mentioned the presentation may initially resemble a variety of medical conditions including acute coronary syndrome, pulmonary embolism or aortic dissection and treatment delay is associated with worse outcomes[48]. A 2013 case series[52] of 120 patients with oesophageal perforation identified 6.8% related to Boerhaave's. Whilst the overall mortality in this series was 11.7% the mortality for the Boerhaave's subset was 33% highlighting the significance of this condition. Kollmar and Lindemann[4] published a case series and literature review. 17 patients presented with Boerhaave's perforation and 6 died (35% mortality) the management was varied and included laparotomy, thoracotomy and various techniques for primary repair or resection. The meta-analysis looked at 18 studies (227 patients). The mortality for patients undergoing primary repair was 13.9%, oesophagectomy or exclusion procedure 19.6%, drainage or conservative management 32.4%. The 18 studies included in this meta-analysis were all small retrospective studies. The authors analysed the effect of a >24 hour delay in surgery and identified that delay in treatment was more likely to result in resection rather than primary repair. The numbers in this sub cohort are small and no control group exists to compare, there is also no way to control for any bias in patient selection or to control for the heterogeneous nature of the studies included in this review.

1.3.6 Anastomotic Leaks

Surgery to the oesophagus for malignancy or injury typically involves removing a section of the oesophagus, its associated blood supply and lymphatic tissue and replacing it with a native tissue conduit. The Ivor-Lewis oesophagogastrectomy is commonly performed and is a two stage procedure to fashion a conduit from the stomach that is brought up into the chest and anastomosed to the oesophagus[53]. A feared complication of resectional surgery is anastomotic leak occurring at the level of the gastro-oesophageal anastomosis, a complication carrying significant mortality and morbidity. Leak rates of 5-20% are reported however many do not require invasive management[54]. Management options mirror those for other oesophageal perforations and range from supportive care including nutrition, antibiotics and keeping the patient nil by mouth to surgical revision of the anastomosis,

cervical oesophagostomy, stents and negative pressure therapy have also been used.

1.3.7 Foreign Body

Ingested foreign bodies are a rare cause of oesophageal perforations[3] but are occasionally seen in by children or patients with a psychiatric history[55]. A 2015 review of 40 studies detailing 168 patients described the predominant symptoms of chest pain, odynophagia and dysphagia[9]. A review of 5 848 patients presenting with foreign body ingestion identified only 8 patients with cervical perforation[56]. Small objects, less than 2 cm in size, will typically pass into the stomach and from there will usually pass through the gastrointestinal tract[57].

1.3.8 Caustic Perforations

Ingestion of corrosive substances can cause significant injury to the oesophagus and stomach in some cases leading to perforation. These injuries are rare but often devastating and case series typically identify only a handful of patients presenting in any given year [58, 59]. A post-mortem study from Turkey identified a 0.089% incidence over a 10 year period [60].

Typically this mechanism affects children who accidentally ingest caustic substances or adults who are attempting suicide [60]. Historically it has been suggested that acids tended to cause injury to the stomach while alkali caused more damage to the oesophagus [61], two prospective studies [62, 63] looking at acid and alkali ingestion respectively failed to confirm this idea and more recent literature [58] also failed to show a difference in injury pattern. Current endoscopic management and high quality critical care has reduced the early mortality following caustic ingestion[64].

Mechanism	Incidence	Mortality and Morbidity
Iatrogenic	0.5%-5%	Low (due to early recognition)
Traumatic	6%-29%	High (due to associated injuries)
Spontaneous	6.8%-15%	High (due to delay in presentation)
Anastomotic leaks	10%-15%	Moderate (variable extent of injury)
Foreign body	<1%	Low
Caustic Ingestion	<1%	Low mortality but high morbidity

Table 1: Summary table of the mechanisms, incidence, and a qualitative assessment of mortality and morbidity obtained from a range of different clinical reports detailed in the main text.

1.4 What investigations are used in the diagnosis and management of oesophageal injuries?

Investigation of injuries to the oesophagus should be multimodal. The mainstays include contrast swallow, computerized tomography, endoscopy and surgical exploration.

Radiographic imaging of the upper gastrointestinal tract is well established. Gastrografin or barium is used as the contrast media although these will miss 10%-30% of perforations [51, 65]; furthermore gastrografin can cause severe necrotizing pneumonitis if aspirated into the lungs while barium can cause fibrosing mediastinitus if it extrasavates [3]. Computerized tomography (CT) is readily available in modern acute care hospitals and can be performed with oral contrast. CT findings of oesophageal perforation with associated mediastinitis include oesophageal thickening, extra luminal gas and contrast media, pleural effusion or abscesses [51, 65]. Plain radiographs have a limited role in the diagnosis of oesophageal perforations. Chest radiographs may show mediastinal air associated with a pleural effusion. A characteristic V sign

has been described [66] however with limited sensitivity plain radiography offers little benefit over CT.

Endoscopy has the advantage of being both diagnostic and therapeutic in the management of oesophageal perforations[12]. For traumatic injuries sensitivity and specificity are over 95% in recent studies[67]. Limitations of endoscopy include logistical considerations related to equipment, the requirement for highly trained staff to perform the investigation and the risks of complications of endoscopy including making the injury worse.

1.5 What are the management priorities and what techniques can be used in oesophageal perforations?

1.5.1 Surgery

Management of oesophageal injuries depends on the condition of the patient and the underlying cause of the perforation[11]. The treatment priorities are to seal the perforation, drain any contamination, treat infection and maintain nutrition[68].

Current treatment options include surgery, stents, negative pressure therapy and endoscopic treatments (Figure 1.3). Surgery has in the past been considered the gold standard for the management of complex perforations[18, 69], however over the last 20 years less invasive treatment options have been investigated and many institutions now have treatment protocols that include endoscopic management for selected patients[3, 6, 8, 52, 67, 70, 71]. The evidence supporting this practice is limited to case series and uncontrolled trials and is contradictory with several studies supporting surgery over non-operative techniques[72] while other studies support the use of endoscopically placed covered stents[73], luminal negative pressure therapy[74] or primary repair with endoscopic clips[75]. To illustrate the variation in clinical practice four important studies are discussed below.

Schweigert and Beattie[76] performed a non-randomised retrospective study comparing outcomes for Boerhaave's Syndrome between two specialist centres in Germany and the United Kingdom. In the UK operative intervention

was the standard approach while in Germany endoscopic stenting was preferred. 33 patients underwent either surgery or stenting, 20 had surgery and 1 patient died, 13 were stented and 2 died. 11/13 patients treated with a stent needed further surgical intervention to drain sepsis. The authors concluded that stents offered no advantage over surgical management and were associated with increased mortality and morbidity.

Hasimoto et al.[72] performed a systematic review comparing surgery with conservative therapy. 33 case series were identified including 1417 patients of which over half had iatrogenic injuries, two-thirds had surgical management one-third were treated conservatively. Mortality in the surgical cohort was 16.3% versus 21.2% in the conservatively managed cohort ($p<0.05$). This study however included surgical drainage procedures in the conservative cohort including open thoracotomy and laparotomy which carry mortality risks in themselves.

A retrospective review of 66 patients with thoracic oesophageal perforation with a range of aetiology including iatrogenic, trauma and Boerhaave's identified the importance of differentiating contained perforations which were managed conservatively with no deaths and no treatment failures from uncontained perforations. The uncontained perforations defined as having a large mediastinal collection with uncontrolled sepsis were managed with primary surgery with a mortality of 7.7% (3/39 patients) while stenting had a mortality of 55.6% (5/9 patients). This was not a randomized study and the stent group was treated on the basis of refusal of surgery. This study also analyzed outcomes on the on the basis of time to intervention concluding that outcomes for surgery were better if performed within 48 hours of the injury [13].

A multicentre European study looked at 194 consecutive patients with oesophageal perforation [77]. 30 day mortality was 17.5% however there was considerable heterogeneity in the baseline characteristics of the patients. The aim of the study was to compare surgery against stent grafting although the patient series included non-operative management and endoscopic clipping among the treatments. Surgery included oesophagectomy, closure over drain,

simple repair and patch repair. No definitive benefit of surgery over stenting was demonstrated.

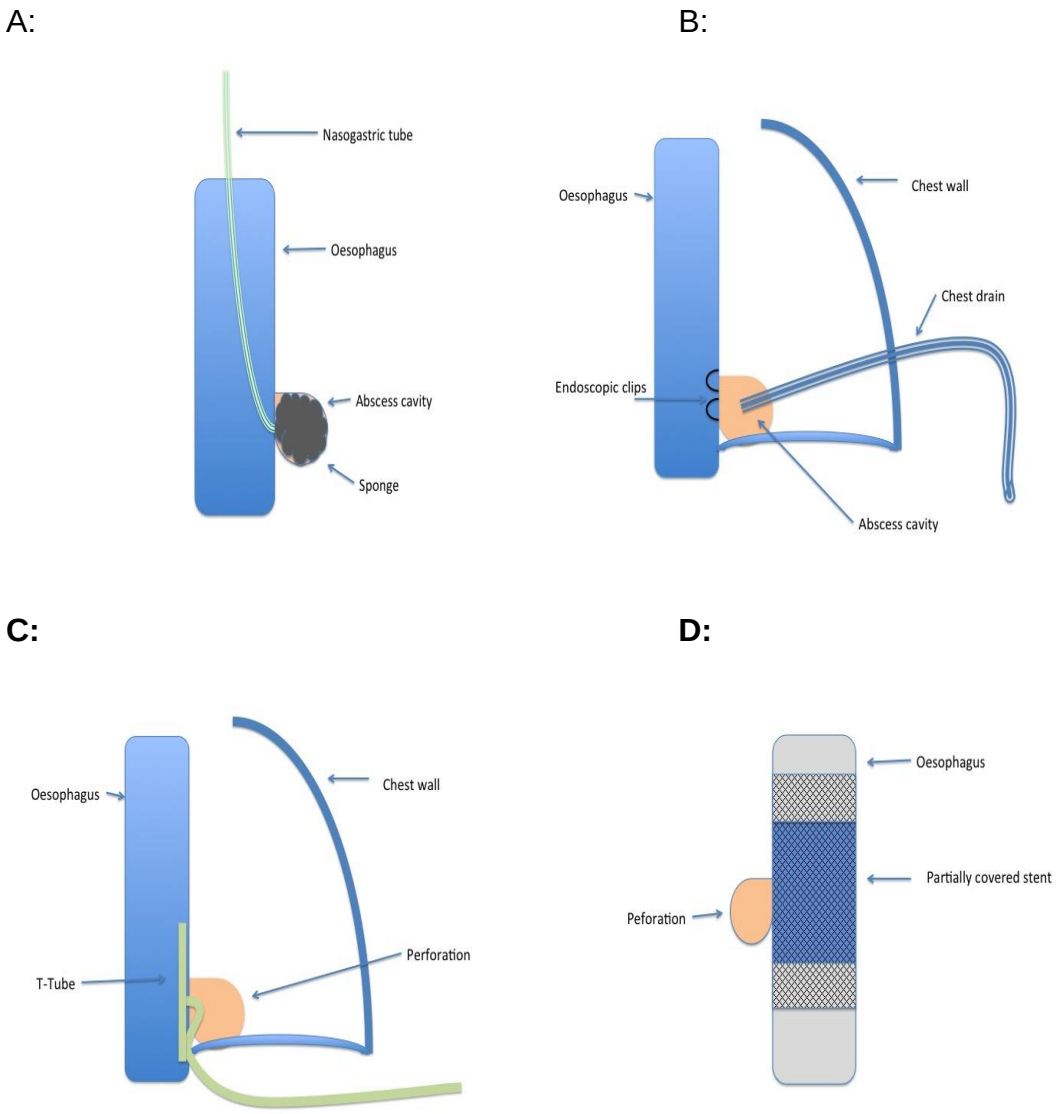


Figure 1.3: Perforation repair techniques a: Endoluminal negative pressure b: Endoscopic clips with external drainage of collection c: T-Tube repair of oesophagus d: Partially covered self expanding metal stent (pSEMS)

1.5.2 Vacuum Therapy

Topical negative pressure therapy is widely used in the management of complex superficial wounds. The effect of this therapy is to draw fluid away from the wound so reducing bacterial load within the wound and to stimulate

healing tissues to create healthy granulation tissue[78]. Endoluminal negative pressure therapy involves placing an appropriately sized polyurethane sponge attached to a nasogastric tube in the perforation. The sponge is changed every 3-4 days until the cavity is small enough to be left to heal unaided[79]. Figure 1.4 shows a pictorial representation.

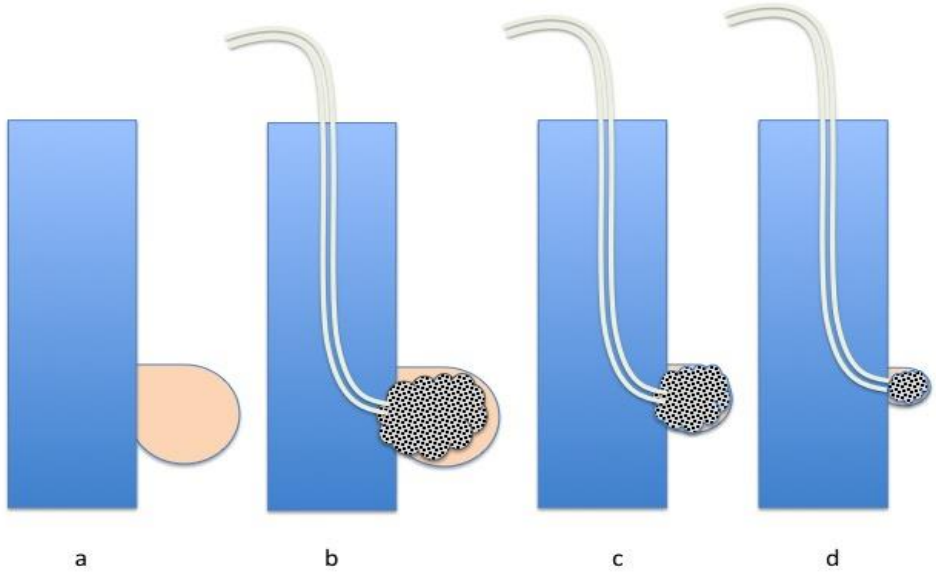


Figure 1.4: Endoluminal topical negative pressure therapy a: Oesophegeal perforation with associated abscess b: the polyurethane sponge is attached to a nasogastric tube and placed in the perforation c & d: as the perforation heals the sponge is replaced for a smaller one every 3-4 days.

Reference	Patients (N)	Indication	Outcome, time to healing	Mortality
Schorsch [80]	35	21 leak 7 iatrogenic 7 other	32/34 healed, median time 11 days	90 day mortality 5.7%
Bludau [81]	14	3 spontaneous 2 iatrogenic 9 post operative leaks	12/14 healed, mean 12 days	14.2% mortality, stents used in conjunction
Heits [17]	10	4 Iatrogenic 5 spontaneous 1 foreign body	9/10 healed, mean 19 days	10% mortality
Schniewind [82]	17	Anastomotic leaks	17/17 healed, not specified	12% mortality
Brangewitz [78]	32	30 anastomotic leaks 1 spontaneous 1 iatrogenic	27/32 healed, mean 23 days	9.4% mortality
Kuehn [83]	9	5 anastomotic leaks 4 perforation	8/9 healed, mean 18 days	11.% mortality
Wedemeyer [84]	8	8 anastomotic leaks	7/8 healed mean 23 days	0% mortality Prospective study
Weidenhagen [79]	6	Resistant leaks	6/6 healed, median 20 days	16.7% mortality
	Total 131		Mean healing time = 19 days	Average mortality 10%

Table 2: Studies of endoluminal negative pressure therapy.

Several studies have looked at the use of endoluminal negative pressure therapy for the management of oesophageal perforations [17, 79, 81, 82], summarised in table 2. These studies are small case series with numbers ranging from 6-35 patients with predominantly retrospective analyses. Brangewitz et al.[78]compared stenting with negative pressure therapy demonstrating a clear benefit in favour of negative pressure, this was not, however, a controlled trial but a retrospective review. Overall this therapy seems to compare well with both operative and endoscopic treatment modalities however no high-level evidence is available.

1.5.3 Stents

Stents can be placed in the oesophagus using a combination of endoscopy and fluoroscopy[85]. The stent must close the defect, provide a watertight seal

and be removable[69]. Self expanding metal stents (SEMS) and self expanding plastic stents (SEPS) are used and these can be fully or partially covered[86].

Stents used to manage perforations can be partially covered or completely covered[16]. Completely covered stents are at risk of migration while partially covered stents are fixed in place due to endothelial ingrowth through the uncovered parts of the stent which can make the stent difficult to remove[87].

The use of stents in the management of oesophageal perforations is well established although in the context of complex perforations with associated contamination and infection surgery is often the preferred treatment[69]. Even when stents are used additional drainage procedures are often needed, a series of 63 patients with post-operative anastomotic leaks were managed with self expanding metal stents (SEMS) and 56% needed additional surgical procedures to manage chest sepsis[88].

Disadvantages of using stents include tissue ingrowth, stenosis, migration, stent fracture, bleeding and perforation [89]. Freeman et al.[90] analysed stent treatment failures in a large cohort of patients with stents for oesophageal leaks. The retrospective analysis suggested cervical and gastro-oesophageal junction location, length greater than 6 cm and a distal conduit leak all contributed to treatment failure although only 15 patients were affected.

Many of the studies looking at the use of stents have been limited to cohort studies [69]. Dasari et al. 2014[73] looked at published case series from 1999-2011. The overall successful occlusion rate was 81%-85%. Plastic stents were more likely to migrate while metal stents were more likely to cause strictures. The authors comment that when combined with suitable management of mediastinal sepsis stents were an appropriate treatment. Studies have identified the lack of randomized studies as a shortcoming with regards making practice recommendations.

1.5.4 Endoscopic Clips

Tissue clips applied via an endoscope can be used to close iatrogenic perforations or secure stents to prevent migration. Clips that pass through the instrument channel of an endoscope are known as Through The Scope Clips (TTSC) while clips that attach to the end of the endoscope via a transparent cap are known as Over The Scope Clips (OTSC) [11].

TTSCs were originally designed for the treatment of gastrointestinal bleeding and need to be small enough to pass down the instrument channel. OTSCs are larger and better suited to closing perforations. Typically clips of either type can be used in the acute setting at the time of injury [91, 92]. Case reports describe the successful use of endoscopic clips in post-operative perforations [93, 94] and spontaneous perforations [95, 96].

Clips can also be used in situations where stents would be inappropriate such as the very proximal cervical oesophagus and at the gastrooesophageal junction [97]. The success rate for acute perforations is 90% but lower for post operative leaks 68% and fistulae 59% [11].

1.5.5 Adhesives

The use of adhesives to treat oesophageal perforations is discussed in the medical literature as far back as 1966 [98]. There are few papers published in the English language and these are limited to case reports [99-102]. A case series from Germany reported 55 patients treated for anastomotic leak (N=46) and oesophageal perforation (N=9) with endoscopic washout and fibrin glue over a 9-year period. 50% of the patients showed signs of sepsis at the start of treatment and all survived [103]. The use of fibrin glue to reinforce a sutured repair of the oesophagus has also been described with positive results in limited human and animal studies[104].

1.6 Ideal Solution

The ideal solution would allow a perforation to be sealed using a minimally invasive technique, for instance endoscopic placement, it would require a single intervention, the closure system would have minimal associated

morbidity compared to current techniques and would not adversely affect healing of the oesophagus. Once the oesophagus had healed the sealing system would be removed from the body or left to degrade naturally leaving a fully functional oesophagus with minimal fibrosis. None of the currently available techniques for oesophageal repair, replacement or regeneration are able to meet all these criteria. A patch that seals a perforation, staying in place until the perforation has healed and then degrades may offer improved treatment outcomes but the patch's physicochemical properties and adhesion mechanism will be central to successful outcome. Alternatively, the patch adhesive could be designed to allow the patch to detach (post healing) and pass through the digestive tract. If the patch were to slough off and pass into the GI tract this should ideally happen after four to six weeks based on the time the oesophagus takes to heal. In this situation the patch would not need to be degradable but would need to have an inert or positive therapeutic effect on local tissues for the duration it was adherent. Table 3 summarises these properties and suggests current solutions.

Feature	Characteristic	Solution
Low toxicity	Patch material and degradation products are not locally or systemically toxic.	polyglycolic acid[105] polylactic acid
No fibrosis	Patch will not promote fibrosis and stenosis.	Drug eluting vascular stents[106]
Degradable	Patch will degrade or detach 6 weeks.	polydioxanone polyglactin 910
Endoscopic placement	Patch can be placed laparoscopically.	Stent placement[16]
Human approved	Previous applications.	polyglactin 910 polydioxanone

Table 3: Summary of characteristics of ideal solution management of oesophageal perforations.

1.7 Biomaterials and tissue engineering/regeneration

The proposed patch would be a biomaterial that would act as a scaffold to support to tissue growth and regeneration. This biomaterial should have properties that allow it to interact with host tissues in a therapeutic manner; this might involve promoting the growth of healthy and normal tissue or restricting pathological processes, in the context of the oesophagus this would be fibrosis that could result in stricture formation.

The term tissue engineering covers a range of techniques and concepts often combining biology and engineering to create functional tissues. In 1993 Langer and Vacanti[107] defined tissue engineering as:

"an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function".

This broad definition has been refined and tissue engineering and tissue regeneration are terms now used to describe the combination of cellular tissue components and non-cellular components to new functional tissue.

"*Tissue engineering is the creation of new tissue for the therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells through a systematic combination of molecular and mechanical signals."*[108]

The non-cellular component is either decellularised biological extra-cellular matrix (ECM) or consists of synthetic materials. Both provide mechanical support, prevent anoikis, guide cell behaviour and support the growth of functional tissues. Research groups have investigated various approaches to oesophageal tissue regeneration. Bacterial cellulose scaffolds have been trialled in rabbit models[109, 110], decellularised small intestine submucosa has been trialled in canine and porcine models[111, 112] and porcine decellularised scaffolds have been trialled in rabbits[113] additionally a variety of synthetic degradable and non-degradable scaffolds have been investigated[114-117].

Several research groups have investigated seeding decellularised scaffolds with autologous cells primarily epithelial cells and muscle cells, with the aim that these will either become part of the new tissue or encourage new tissue growth through the release of growth factors. As the oesophagus is composed of many cell types (endothelial, neurones, smooth muscle) the use of adult stem cells has also been investigated with the hope that these cells will differentiate into these multiple cell types. Spurrier et al.[118] used oesophageal organoids, small sections of oesophagus of several cell types, derived from mice. The organoids were cultured on polymer scaffolds and implanted into a mouse model. Subsequent histological examination demonstrated differentiation of the mesenchymal cells into nerve, muscle and epithelium using Green Fluorescent Protein (GFP) labelling to track the fate of the organoids. While many researchers are looking at creating a fully tissue engineered oesophagus similar techniques have been used to repair small defects akin to the perforations seen in clinical practice. Zhu et al.[109] used bacterial cellulose patches in a rabbit model while Lynen et al.[119] used degradable and non-degradable commercial surgical mesh to repair 5mmx10mm defects in the oesophagus of rabbits. A number of researchers have used engineered cell sheets to replace epithelium following surgical excision of malignancy in human trials[120] .

1.7.1 Biomaterials

Materials used in biological applications must be designed to support desirable biological interactions, which includes the cellular (local or systemic) response to any dissolution or breakdown products from the material. These desirable biological interactions are often described by the term “biocompatibility”, which can be defined as the ability of a material to interact appropriately with its surrounding environment[121], although Williams[122] argues that biomaterials should be considered in the context of the system they interact with.

The challenge in developing biomaterials is that different applications require different interactions and thereby different material properties. Biomaterials that are in contact with blood must be able to resist thrombosis[123] and many

temporary plastic devices such as urinary catheters must resist biofilm formation[124]. Biomaterials also need to encourage desirable protein and cell interactions in order to promote functional tissue formation, and/or prevent adverse biological outcomes such as scarring or toxicity. Biomaterials can be manufactured from a wide range of materials (including ceramics, metals, biological polymers and synthetic polymers) depending upon the structural support, size and functionality required. One of the most tailorable materials in terms of surface chemistry, degradation rates and mechanical properties are polymers.

1.7.2 Polymers

Polymers are extensively used in biomedical applications with polyurethanes particularly suitable due to their tailorable biomechanical properties, surface chemistry and fabrication methods [105]. Since their discovery in 1937 by Otto Bayer, applications have included pacemaker leads[125], catheters, vascular grafts and prosthetic heart valves[126] while experimental applications include tissue and organ regeneration[127, 128].

1.7.3 Polycaprolactone

Polycaprolactone (PCL) is a polyester which degrades by hydrolysis under physiological conditions resulting in non-toxic by-products. The degradation rate can be varied by altering both the constituents of the polymer and the polymer surface exposed to water by fabricating different porosities and structures[129]. If water can permeate evenly throughout the 3D structure of the polymer then hydrolysis can occur throughout resulting in bulk degradation, conversely if water can only interact with the exterior surface then surface erosion will occur. Hydrolysis is catalysed in the presence of acids and bases and the presence of acidic breakdown products results in autolysis as the degradation products further catalyse the degradation[129, 130]. In the presence of water the PCL degrades to Acetyl-CoA which enters the citric acid cycle producing water and CO_2 as shown in figure 1.5.

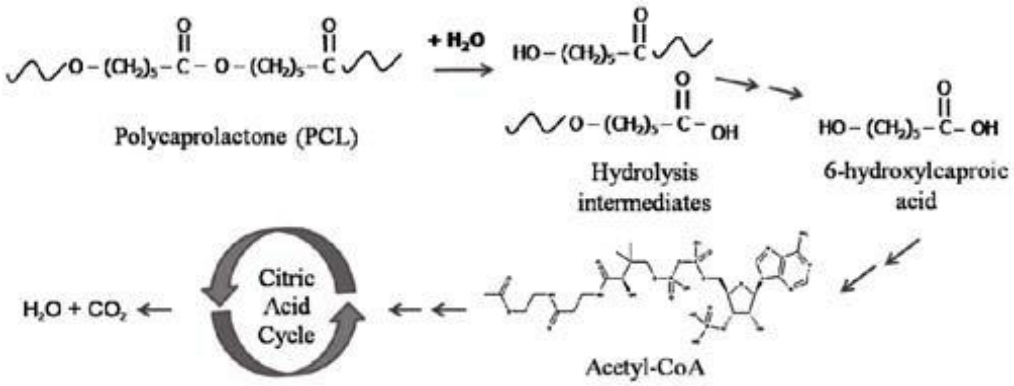


Figure 1.5: Hydrolysis of PCL with intermediate steps and degradation products.

1.7.4 POSS

Polyhedral oligomeric silsesquioxane (POSS) was developed for biomedical applications in the 1960s and is now used in a wide variety of applications[131]. POSS can be used to create nanocomposite materials with properties that allow even greater control of the material physicochemical properties that enables tailored materials for specific applications[132, 133].

1.7.5 POSS PCLU

POSS PCLU is a propriety polymer consisting of polyhedral oligomeric silsesquioxane (POSS) nanoparticles suspended in poly(e-caprolactone urea) (PCLU) and was developed as a degradable evolution of a similar POSS containing polyurethane POSS PCU (UCL-Nano™)[134]. Its properties and applications have been extensively investigated as part of previous work within this laboratory[105, 135-137].

POSS PCU has been used for tear duct replacement[138] while vascular grafts and synthetic scaffolds for tissue regeneration have been investigated in vitro[137, 139, 140]. POSS PCLU has been studied in the context of skin tissue engineering, vascular graft and paediatric applications[141, 142].

POSS PCLU has been proposed for a number of biological applications due to its physical properties, relatively slow degradation rate compared to polyglycolic acid (PGA) or polylactic acid (PLA) and promising biological interactions[105].

1.8 Surface modification of polymers

Materials used in biological applications must be designed to cause desirable biological interactions, which includes the effect of any dissolution or breakdown products from the material[143]. Material-biological interactions are governed by properties of the material including surface chemistry, mechanical properties (macro and substrate stiffness) and released products (designed and non-designed). The surface chemistry of a material will determine the protein adsorption, which in turn will influence cell attachment and the behaviour of cells growing on that surface[144-146]. Modifying the surface of the material can, therefore, modify initial biological interactions which, in turn, can affect the duration of inflammatory response, fibrotic encapsulation or intimate contact with surrounding tissues. Surface modification of materials can modify biological responses and include topographical, mechanical and chemical alterations. Chemical modifications can include, acid or alkali etching, wet chemistry modification, Ultra Violet light, electron radiation or plasma treatment[121, 147, 148].

Plasma is a partially ionised gas where electrons have been added to or removed from a molecule giving it a charge. This can be achieved using very high temperatures (in the range 4000-20000K) and while these conditions may be suitable in the manufacture of ceramics and metals they are clearly not applicable to biopolymers. Passing high voltage across a carrier gas such as oxygen can create low temperature plasmas[124]. The resultant low temperature plasma can be used to clean, modify and etch polymer surfaces.

Plasma treatment has been reported to improve the adhesion of various biologically active molecules (amine, carboxyl, hydroxyl) to the surface compared with other techniques such as wet chemistry modification or ultraviolet light treatment and has the advantage of minimal temperature change and is suitable for materials that can resist vacuum,[121, 149, 150].

Low temperature oxygen plasma treatment has been used to modify POSS PCU, a non-degradable polyurethane[151, 152] while other research groups have

looked at the effect of plasma surface modification on a range of biomaterials including poly (L-Lactide)[144], PCL[145, 146, 153] PTFE and Dacron[123]. Looking in more detail at this work, Chaves et al.[154] used allylamine surface modification to add amino groups to the surface of the polymer which not only improved cell attachment in short term cell culture experiments but also facilitated osteogenic differentiation of adipose derived stem cells. Similarly Griffin et al.[151] used plasma modification to add amino and carboxyl groups to the surface of POSS PCU which increased the hydrophillicity of the polymer and increased protein adsorption without effecting the bulk mechanical properties.

Surface properties of biomaterials can be assessed in a number of ways. Atomic force microscopy can be used to measure the stiffness of a non-conducting material and topography[155]. Fourier Transform Infrared Spectroscopy (FTIR) can be used to identify the biochemistry of a material surface[156] while water contact angle measurements can measure wettability[153, 157]. The wettability can determine protein attachment and thereby cell interactions. As fibrin glue is based on protein interactions, the surface chemistry is likely to influence these interactions[158].

1.9 Infection

Any material implanted into a biological system can introduce or be a focus of infection[159]. Typically implants that become infected must be removed as natural immune mechanisms and systemic antimicrobial therapies are ineffective in this setting. Materials have been developed aiming to be more resistant to infection either through modification of the material to resist biofilm formation or the introduction of antimicrobial agents in the material itself[160]. Synthetic implants that leach antimicrobial agents are used in clinical practice to treat or prevent infections[161]. Gastrointestinal stents carry a risk of recognised complications as described previously[87], infection tends to associated with perforation although stents will be colonised soon after insertion due to their intraluminal position. In the context the oesophageal patch proposed in this study the patch would be sitting in the lumen of the oesophagus an area that is routinely exposed to bacteria although initial immune protective mechanisms are in present. Strategies to reduce the risk of

infective complications of the patch would include using a degradable material that will not act as a long term focus of infection or using a patch that detaches from the injury site once healing has been achieved again not offering a focus of infection. Surface modification and the addition of anti-microbial agents may also be beneficial in reducing the risk and impact of infection[106].

1.10 Adhesives

Adhesives have a wide application in medicine from the use of cyanoacrylate glues in surgery for wound closure to bone cements used in joint replacement surgery. In the setting of biomaterials and tissue engineering adhesives play an important role at the interface between biological systems and engineered substrates. Adhesives offer a number advantages over sutures or staples: they cause less pain, do not leave a foreign body that may be a nidus of infection and are quicker to apply[162]. Adhesives, however, need to not have undesirable local or systemic effects; they must not prevent normal tissue function and need to provide adhesion strong enough to support the function of the tissue/scaffold construct until the tissues have regenerated sufficiently to render the adhesive unnecessary. There are several types of adhesives that vary in their mechanical strength, degradation rate and toxicity of dissolution products.

1.10.1 Cyanoacrylates

Cyanoacrylate glues are familiar to many as they form the basis of superglues; patented in 1942 these glues have a wide range of uses. Cyanoacrylates were used in vetinary practice in the 1970s and commercial superglue was used in humans in pre-hospital settings in the Vietnam War. Medical cyanoacrylates have been in use since the 1970s however their use was limited due to concerns about skin irritation and tissue toxicity related to the breakdown product formaldehyde[137, 163].

Bornemisza et al.[164] used a cyanoacrylate glue to repair experimental oesophageal perforations in a canine model combining the glue with collagen patches or a cellulose based scaffold and comparing the efficacy of closure

against sutured repair. All animals in the glue groups survived while half the animals in the sutured group died.

Human intra-corporeal use of cyanoacrylates has been limited due to concerns about the safety of the breakdown products and as a result cyanoacrylate glues used in-vivo are most commonly limited to external applications only. In 1998 2-Octyl cyanoacrylate was approved by the United States Food and Drug Administration for medical use under the trade name Dermabond. This formulation is less brittle than previous formulations and as such made a more effective would dressing[165]. Cyanoacrylates are now widely used in medical applications for wound closure replacing traditional suturing techniques.

1.10.2 Semi-synthetic glues

A number of semisynthetic adhesives have been developed exploiting the properties of biological gels and polymers. Gelatin in combination with formaldehyde, resorcinol and gluteraldehyde (GRF glue) has been used in the management of aortic dissection[166]. Gelatin has also been polymerised with a variety of cross linking agents to produce biologically useful adhesives[167]. Vuocolo et al. used a photochemical process to polymerise gelatin for use as an adhesive/sealant to reinforce gastrointestinal anastomoses; burst testing withstood pressures up 8kPa with no evidence of direct toxicity or inflammatory reaction[168].

Albumin can be polymerised using gluteraldehyde as a crosslinking agent and is used in the surgical repair of aortic dissections and in cardiac and vascular surgery as a haemostatic agent under the commercial trade name Bioglue (Bioglue®, Cryolife, USA) [169]. Albumin has also been used experimentally combined with tartaric acid to form a biocompatible adhesive[170].

Chondroitin sulphate is a component of cartilage and has been used in combination with polyethylene glycol to create a versatile hydrogel adhesive for wound healing and tissue regeneration[171] while Wang et al. used

chondroitin sulphate functionalised with methacrylate and aldehyde organic groups to bind regenerated cartilage to native cartilage[172].

In the experimental setting many of these glues are synthesised in house using biological components. This is a complex process and there is risk of considerable variation in the final product. The practical application of these two types of adhesive is also problematic as there are risks of blood and tissue borne infections as well significant regulatory hurdles. These risks can be minimised or removed by using commercially available products.

More recently the role of nanoparticles as adhesives has been investigated making use of unique adsorptive properties. Rose et al. [173] investigated a number of nanoparticle solutions and achieved the adhesion of two pieces of calves liver together using a silica nanoparticle solution. The mechanical strength of this bond ranged from 6-25Jm^{-2}.

1.10.3 Fibrin Glues

Fibrin glues or sealants are derived from blood plasma and consist of soluble fibrinogen mixed with thrombin, calcium, Factor XIII and bovine aprotinin to create an insoluble polymer of fibrin. Fibrinogen is hydrolysed in the presence of thrombin to form fibrin monomers that then polymerise in the presence of Calcium ions. Thrombin, in the presence of Calcium, catalyses Factor XIII to Factor XIIIa which produces covalent cross links between alpha and gamma chains of fibrin monomers forming a three dimensional matrix[174](Figure 1.6).

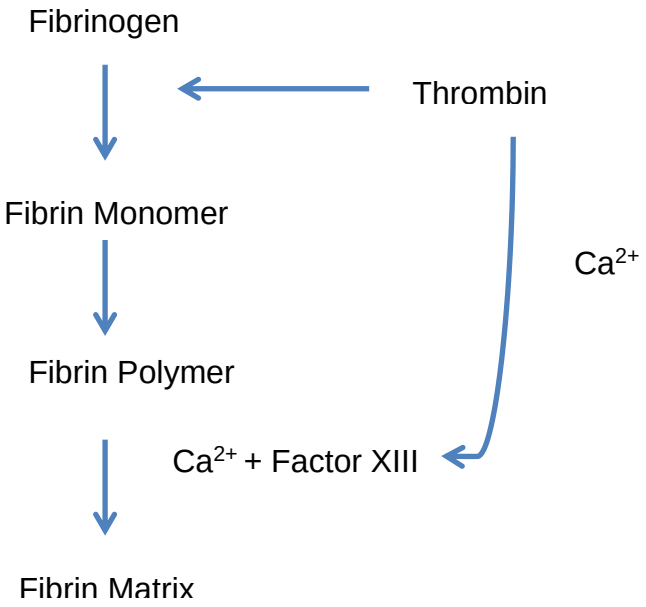


Figure 1.6: Mechanism of fibrin clot formation (adapted from Suzuki et al.[174])

Fibrin polymer matrix adsorbs to the polymer surface and binds via RGD moieties on Integrins on cell and extracellular matrix surfaces (Figure 1.7). Platelet aggregation is an important, fibrin generated, component of the blood coagulation pathway and is mediated by the αIIβ3 integrin[175]. Work looking at which component of the integrin molecule contributes to fibrin adhesion highlighted the importance of the β_1 subunit[176].

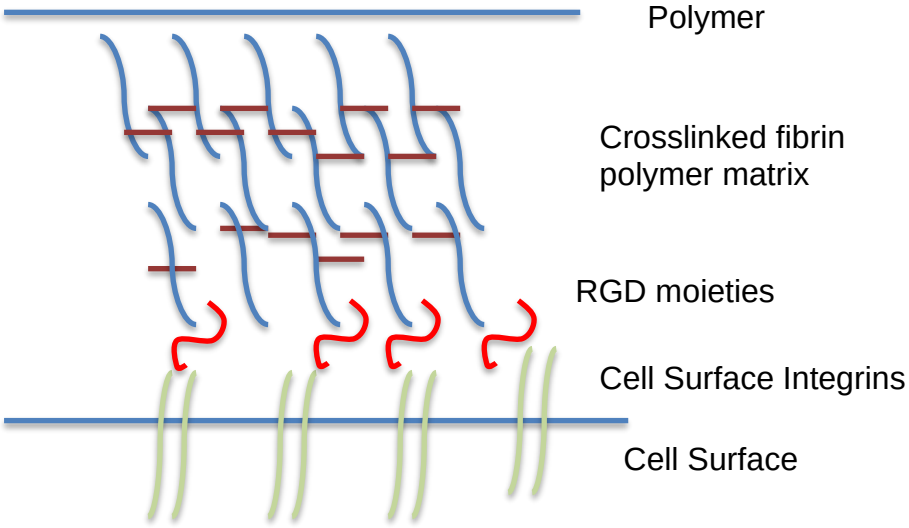


Figure 1.7: Fibrin adhesion between polymer and cell surface.

Fibrin glues are widely used in surgical applications for their adhesive and haemostatic properties and have been used as scaffolds to support tissue regeneration. Town described the use of fibrin in ophthalmic surgery in 1950 [177] and Kort reviewed the uses of fibrin glue in general thoracic surgery in 1966 [98] focusing on oesophageal repair in two other papers [178, 179] in the same year.

Throughout the 1970s and 1980s researchers looked at the role of fibrin glues in bone healing[180], gastrointestinal anastomoses[181], vascular surgery[182], cardiac surgery[183] and ENT surgery[184]. The role of fibrin glue in nerve regeneration acting as a biological scaffold was also investigated[185].

1.10.4 Adhesives in Oesophageal repair

McCarthy et al.[186] used fibrin glue to reinforce sutured repair of an experimental canine model of anastomotic leak. Their results suggested that fibrin glue reduced the rate of a significant leak. Vakalopoulos et al.[187] performed a systematic review of the literature relating to the use of tissue adhesives in a range of gastrointestinal anastomoses. The review looked at human, animal and laboratory based studies and found broadly favourable results however the review notes that human clinical studies were limited in number and applicability. The advantage of using commercially available fibrin glues is in overcoming problems associated with manufacture and maintaining product consistency, infection control and regulatory authority. For these reasons a commercial product is desirable.

1.11 Thesis Aim

The aim of this project is to develop and validate an ex-vivo bench top model of oesophageal perforation in order to test characteristics of a novel patch treatment. Porcine oesophagus was used to create the model and aspects of the material characteristics, validity of the model when assessing repair methods and the utility of the model in testing aspects of the proposed patch was also tested.

1.12 Thesis Outline

The thesis explores a possible solution to oesophageal perforation repair, through the development of a self-adhesive, degradable patch that could be deployed via a flexible endoscope. The patch would seal the oesophageal perforation and remain in situ until the oesophagus had healed allowing normal function of the oesophagus during healing. The patch could be degradable and be broken down within the body or the adhesive could be designed to degrade allowing the patch to pass into the gastrointestinal tract. The patch should not adversely effect healing indeed could be designed to promote healing and reduce stricture formation. To investigate the success of oesophagus perforation patch intervention in comparison to other repair approaches, it was first necessary to develop an ex vivo model of oesophageal injury. This was then followed by the development of the polymer patch, investigation of cell interactions and its attachment to the oesophagus in the ex vivo model. The thesis is divided into chapters each detailing a discrete aspect of the overall project.

Chapter two, Materials and Methods, describes the generic methodological approach and techniques, including the manufacture and properties testing of the polymer patch, surface chemistry modification, surface characterisation assessment and cell-material interaction assessment.

Chapter three covers the development of the ex vivo porcine oesophagus inflation model. The aim of this set of experiments is to define some material properties of the oesophagus and create a model that allows the testing of oesophageal repair techniques.

Chapter four covers the development of the patch itself. The aim of this chapter is to examine the development of the polymer patch, the assessment of stiffness, assessment of wettability, surface chemistry modification in the form of plasma surface modification and cell-material interactions.

Chapter five looks at adhesives and patch adhesion. The aim of this set of experiments is to examine the use of fibrin glue in the context of attaching a polymer patch to oesophageal tissue. The chapter looks at experiments assessing the effect of surface chemistry modification on adhesion as well as the effect of time on adhesion strength.

Chapter six reviews the project and provides a discussion of the results and the existing literature.

2 Materials and Methods

2.1 Introduction

This project covers a number of different areas including material testing, ex vivo model development, cell-material interaction, surface chemistry modification and material adhesion. The following sections give details of the materials and methods used in each series of experiments.

2.2 Inflation Model

2.2.1 Introduction

The oesophageal inflation model required a number of components including an oesophagus, a method of inflation, a pressure sensor, a data-recording device and a means of detecting leaks for the repair and patch testing stages of the project. In surgical practice intraoperative leak testing is performed by submerging the anastomosis in water or saline and then insufflating air into the lumen of the oesophagus near the anastomosis and observing for bubbles.

During therapeutic endoscopy balloons can be inflated within the oesophagus to dilate strictures or treat oesophageal spasm. These balloons are inflated with a pressure-regulated device an example of which is shown in figure 2.1.

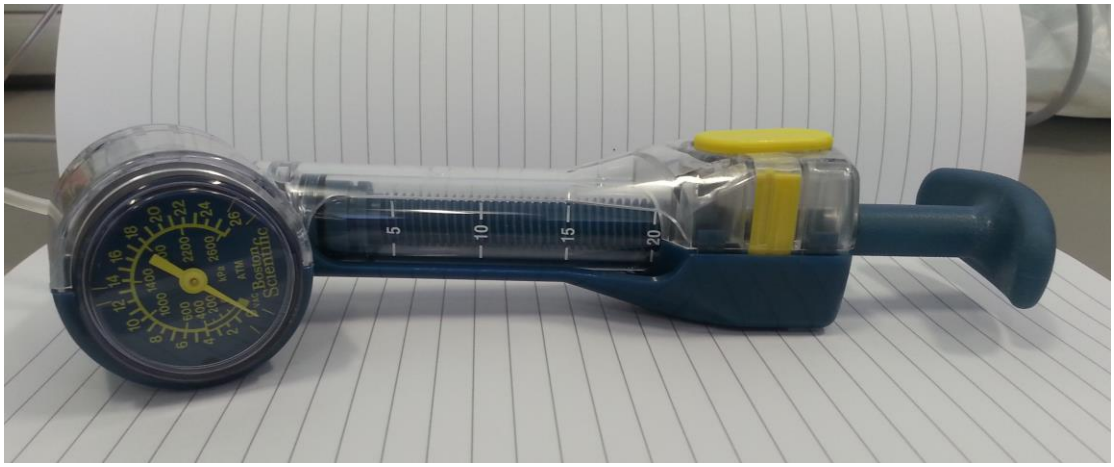


Figure 2.1: Pressure regulated inflation pump (Boston Scientific, Massachusetts, USA).

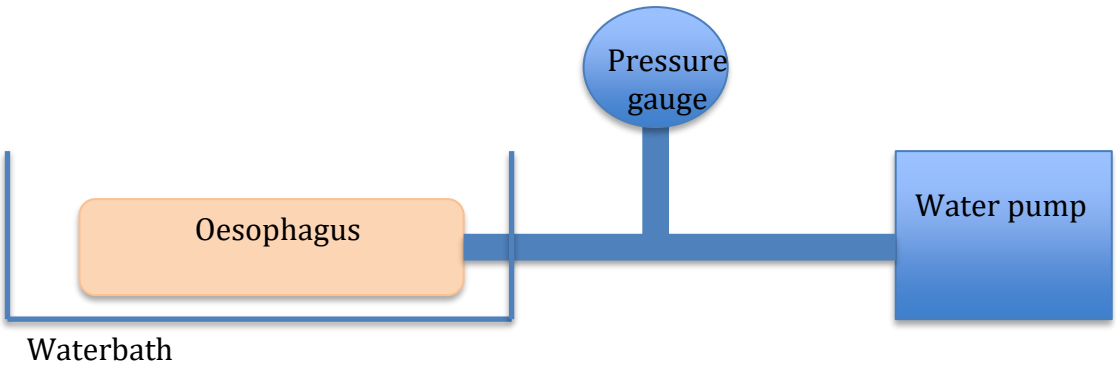
2.2.2 Initial development

The initial model used a balloon inflation pump to insufflate air into the oesophagus the idea being that the balloon pump would allow pressure to be measured while bubbles would be seen at the site of a leak.

Porcine oesophagus was obtained from a commercial medical supplier of animal products (MedMeat), a camcorder (JVC) was used as a data recorder. The oesophagus was placed in a water bath and connected to the pump using silicon tubing. This model failed as the inflation device calibration proved insufficiently sensitive and the device was cumbersome to use. The volume of air in the syringe was insufficient to fully inflate the oesophagus and in re-priming the syringe most of the air escaped.

2.2.3 Refinement

Yang et al. (2006) developed a test rig to examine the material properties of the explanted human oesophagus. They inflated the oesophagus specimens with water and used a digital pressure gauge so a refinement to the model was made by including a variable flow rate water pump instead of the inflation device (Figure 2.2). A three-way tap was introduced into the system to allow a digital pressure gauge (Comark 9500) to be added to the circuit. Flexible silicon tubing was used to connect the pump, pressure gauge and water bath to the oesophagus. The oesophagus was secured to the pump system using cable ties and a large plastic clip secured the distal end allowing the oesophageal specimen to be deflated at the end each inflation cycle (Figure 2.2).



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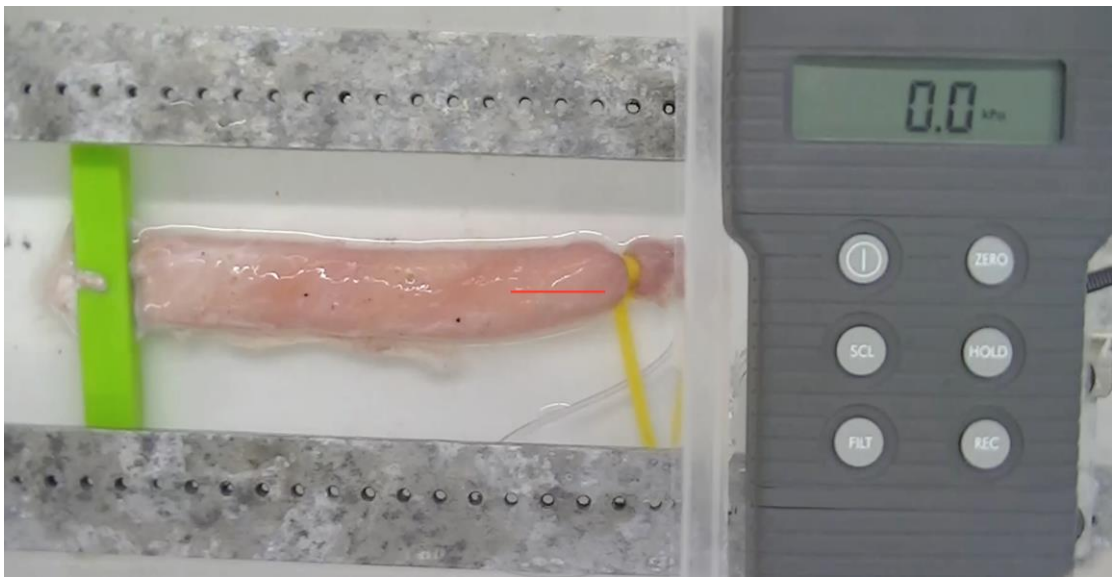

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Figure 2.2 a) Diagrammatic representation of oesophagus inflation test rig showing the key components and their arrangement. b) A still picture from a video recording of an inflation test showing the oesophagus and the digital pressure gauge.

This model reproducibility was assessed, and failure was straightforward to see as water leaked out of the oesophagus. Each inflation cycle was recorded using a digital camcorder and still images were taken at set pressure points. Imaging software was used to measure the diameter of the oesophagus and these data were used to generate stress strain curves. After a series of tests to optimise the model experiments were performed as described in chapter 3.

2.3 Patch Development

2.3.1 Polymer Synthesis

POSS PCLU was synthesised in house following a protocol developed during previous doctoral work[105, 188]. The result of the synthesis protocol was an 18% solution by mass of polymer in dimethylacetamide (DMAC) solvent. This was diluted to 12% by mass to reduce the viscosity of the liquid which facilitated the casting of sheets of polymer. Polycaprolactone (PCL) without POSS was synthesised in the same manner and used as a control polymer in some experiments.

2.3.2 Patch Manufacture

Sheets of POSS PCLU were cast on glass plates using a mold 100mm by 100mm. In order to achieve a specific thickness of polymer the required mass of polymer needed was calculated. The desired mass of polymer was then poured onto the glass plate placed on a balance to allow accurate measurement.

The amount of polymer by weight was calculated from the volume of the polymer sheet 100mm x 100mm x thickness in mm x the density of the liquid (1.15g/l). The glass plate with the liquid polymer was then placed, on a level surface, in an oven at 60^{o}C overnight to facilitate the curing process. Cured polymer sheets were stored between layers of aluminium foil in ambient conditions prior to use.

2.3.3 Material sterilisation

Prior to use in cell culture experiments the polymer needed to be sterilised and endotoxins removed. Sterilisation kills microorganisms but may not necessarily remove the biologically active (inflammatory) endotoxins and therefore both sterilisation and washing needs to occur. Commercial medical materials can be sterilised in a variety of ways including gamma irradiation, autoclaving or ethylene oxide gas. When sterilising delicate materials and biological samples it’s important to consider hoe the sterilisation approach may change the material properties.

Sterilisation techniques for POSS PCLU have been previously investigated [188] and the technique used in this study is an evolution of the results of this study. Ethanol treatment (70% v/v) has been previously shown to modify surface properties of polyurethanes but this sterilisation approach (on the POSS-PCLU polymer) did not significantly influence the mechanical properties or the cellular interactions[134, 189]. For this study polymer samples were sterilised by washing in TWEEN® 20 for one minute (to remove endotoxins) followed by 70% ethanol, with phosphate buffered saline (PBS) washes between. This cycle was completed twice and undertaken in a biosafety cabinet. Samples were not stored but used immediately.

2.4 Cell Culture Experiments

2.4.1 Introduction

Biocompatibility is an important feature of polymers used in biomedical applications however, as discussed in chapter 1, biocompatibility is a property that can only be assessed in the context of the biological system the polymer will exist in. Biocompatibility can be assessed using a variety of techniques including in vivo and in vitro experiments.

In vivo experiments allow the biological interaction between the polymer and the living tissues to be examined over a period of time and can be used to investigate immune reactions, inflammatory responses and toxicity. However animal experimentation is expensive and must satisfy safety and ethical standards.

In vitro experiments examining the effect of a material on cell growth and behaviour are a useful initial starting point in assessing biocompatibility allowing strict control of environmental and experimental variables. Cellular models are considerably cheaper than animal models and allow much closer control. The disadvantages of cellular models are that they only test certain aspects of the biological system such as toxicity and cell adhesion and by using only one cell type these experiments will not fully assess the effects of the polymer on complex tissues. Cell culture techniques were chosen for this

set of experiments as they represented cost effective scientifically valid assessments that are widely used and would allow the results of the experiments to be interpreted alongside other work.

This set of experiments looks at the effect on cell behaviour of POSS PCLU patches with varying properties.

2.4.2 Cell Culture

Human Dermal Fibroblasts are a well-established cell line that are easy to culture using standard techniques, they are readily available and are an important constituent of healing tissues.

Human Dermal Fibroblasts (HDF, obtained from ECCAC and used within passage 8-12) were cryopreserved in dimethyl sulfoxide (DMSO) and foetal bovine serum (FBS) and stored under liquid nitrogen. The cell growth media was Dubecco's Modified Eagle Media (DMEM), 10% FBS (v/v) and 1% (v/v) penicillin-streptomycin antibiotic. Media was refrigerated for storage and warmed to 37^{o}C in a waterbath prior to use. Cryovials of cells were defrosted and added to media in cell culture flasks. These flasks were then placed in incubators at 37^{o}C in a 5% CO_2 atmosphere. The flasks were left for 24 hours to allow cells to attach to the flask wall, the media was then changed and the flasks were inspected under the microscope every day until 80% confluency was achieved. Media was changed every three days.

2.4.3 Cell Passage

In order to have enough cells for to perform the cell behaviour experiments the flasks were split to allow large numbers of cells to be grown. The standard procedure for passaging cells was followed, briefly, when 80% confluency was achieved, assessed visually using light microscopy, the flasks were placed in a biosafety cabinet, the media was removed, the cells were washed with PBS and trypsin (1% v/v) was added to lift the cells off the flask wall. Cell detachment was assessed using light microscopy, and fully supplemented media was added to create a cell suspension which was then centrifuged to separate the cells from the media/trypsin mixture, the resulting cell pellet was

resuspended in fresh media (fully supplemented) and the cell suspension was divided between cell culture flasks.

2.4.4 Cell Counting

For each of the cell culture experiments a defined number of cells were added to multiwell plates. A sample of cell suspension was stained with trypan blue and cells were counted using a haemocytometer. The number of live and dead cells (as determined by membrane permeability allowing trypan blue uptake) in each of the four quadrants was counted and the mean number of cells was calculated. The number of cells per millilitre in the suspension was then calculated using the formula:
2 (dilution factor for trypan blue) x 10^5 (to convert to cells/ml) x N (number cells counted).

This number was then multiplied by the total volume of the cell suspension to give the total number of cells in suspension. Initial growth experiments conducted using Human Dermal Fibroblasts produced approximately 500 000 cells per 75 ml flask at 80% confluency and this was used as the basis for calculating the number of flasks required for each set of experiments.

2.4.5 Cell Behaviour

Cell behaviour is an umbrella term describing cell growth and function. When assessing cell interaction with a polymer cell respiratory activity can be assessed using a metabolic activity assay. This is a simple, cost effective and reliable test that is widely used allowing results of these studies to be compared to other studies. Cell replication can be assessed by establishing the total number of cells. This is done by measuring the total amount of DNA in a sample and calculating the number of cells using a calibration curve. Again this assay is cost effective and reliable and allows results to be compared to other studies. By combining the results of metabolic activity and cell number the metabolic activity per unit of DNA can be determined. By varying certain properties of the polymer patch the effect on cell behaviour can be observed. In this study metabolic activity was assessed using the alamarBlue® (Invitrogen) assay and total DNA was assessed using the DNA

quantitation kit, fluorescence assay (Sigma-Aldrich®) both were used according to manufacturer's instructions.

2.4.6 Metabolic activity

The alamarBlue® Cell Viability Reagent (Invitrogen) was used to determine cell metabolic activity. The active component of alamarBlue® is Resazurin which is reduced to resorufin by metabolically active cells. In its reduced form the resazurin is pink and is highly fluorescent, in its native state it is blue and does not fluoresce. The assay was performed following the manufacturers instructions, briefly alamarBlue® reagent was added to each well in a multi-well plate the volume added being 10% of the volume of the media in the well. The plate was then incubated at 37°C in 5% CO_2 for 4 hours wrapped in foil to prevent light affecting the reagent. The media was transferred to a black 96 well multiwell plate and the fluorescence was measured using an automated plate reader. Excitation wavelength used was 540nm and emission wavelength used was 600nm. The accuracy and sensitivity range of the assay was determined using a serial dilution of fibroblast cell number and fluorescent OD (Figure 3.1).

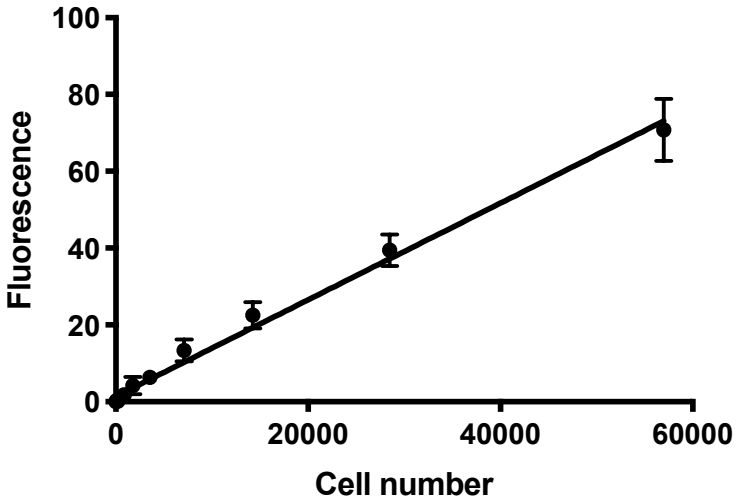


Figure 2.3: Dermal fibroblasts metabolic activity (as determined by alamarBlue® assay) in relation to cell number. N=4, error bars = SD.

2.4.7 Cell number

The metabolic assay gives an average of the activity across all the cells in a population of cells. Results are therefore dependant on both the activity of the cells and the total number of cells. Measuring the total DNA in a system allows the number of cells to be calculated as the amount of DNA per cell is constant.

The DNA quantication kit, fluorescence assay (Sigma-Aldrich®) was performed following manufacturers instructions. In summary, 200µl of sterile DNA free water was added to each well of the 96 well plates used for the alamarBlue® assay, the plates were subjected to six frieze-thaw cycles to lyse the cells. The assay reagent was added following the manufacturers instructions and the fluorescence was measured using an automated plate reader.

Two curves were generated using a serial dilution technique using a known number of cells and a known concentration of DNA. This allowed the fluorescence measurements from the experiments to be converted to total amount of DNA.

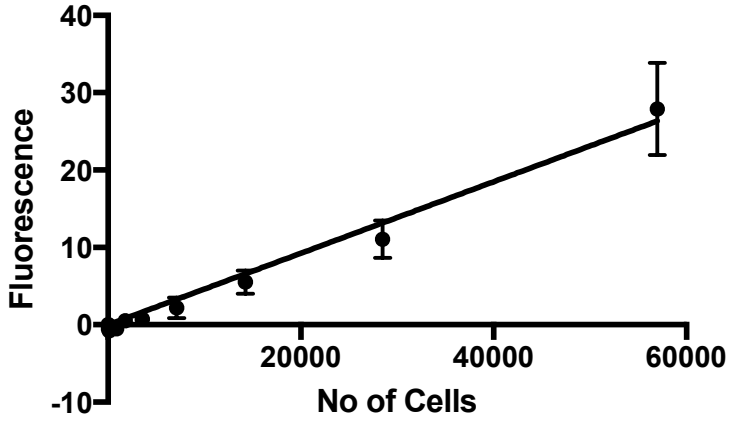


Figure 2.4: totalDNA assay fluorescence sensitivity curve for human dermal fibroblasts. N=4, error bars = SD.

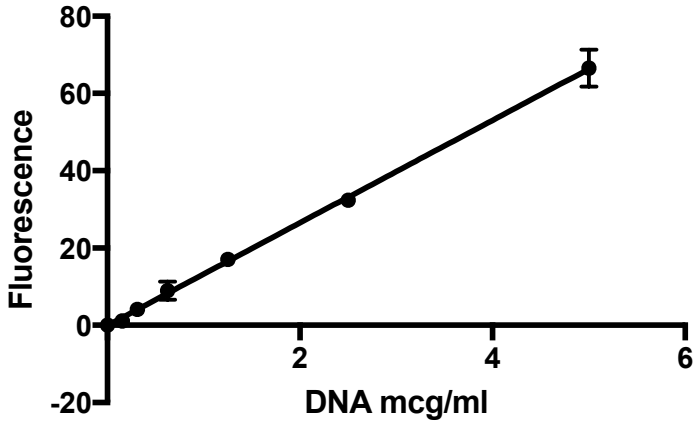


Figure 2.5: Total DNA assay standard fluorescence curve using bovine thymus standard DNA sample. N=4, error bars = SD.

2.4.8 Cell Culture Experiments

All cell culture experiments followed a standard procedure, following passage and splitting as described above 24 well plates were prepared with the polymer discs and Tissue Culture Plastic was used as the control. Experiments lasted for seven days and metabolic activity and cell number were assessed at 24 hours, 72 hours and 96 hours. Each well of the plate was seeded at a density of 10000 cells per cm. All analysis was carried out in a biosafety cabinet under aseptic conditions and metabolic activity and total DNA was calculated as described in sections 2.4.6 and 2.4.7 respectively.

2.5 Adhesion Testing

2.5.1 Strength of fibrin adhesive

Using fibrin glue to attach materials to tissues is a well-established technique[190-192], but has not previously been used for attaching polymer materials to the oesophagus. Initially porcine oesophagus was cut into 20mm by 50mm sections with corresponding POSS PCLU patches of the same size. Commercially available fibrin glue (TISSEEL™, Baxter Healthcare Corporation) was used to attach polymer to tissue with a 20mm by 20mm overlap. The adhesive strength was tested using the Instron Universal testing system after allowing the glue to polymerise for 30 minutes.

2.5.2 Effect of surface modification on fibrin strength

Surface chemistry of the polymer was modified using cold oxygen plasma as described in section 2.6. Modified polymer patches 20mm x 50mm x 150µm were attached using TISSEEL™ fibrin glue with 20mm x 20mm x 150µm overlap. The glue was allowed to cure for 30 minutes and the attachment strength was measured using the Instron Universal testing system as detailed in Section 5.2.

2.5.3 Effect of media on fibrin strength

One of the issues with fibrin glue is the degradation rate. This study investigated the strength of the fibrin glue attachment (polymer to polymer) after a week in standard laboratory media (37^{o}C) aiming to simulate an in vivo environment. Polymer patch (20mm x 40mm x 150µm) rectangles of were cut from cast sheets using a laser cutter. The pieces of polymer were stuck together using TISSEEL™ with a 20mm overlap. The adhesive strength was tested using the Instron Universal testing system after allowing the glue to polymerise for 30 minutes.

2.6 Material Testing

2.6.1 Biaxial mechanical testing of polymer patch

Having established a robust and reliable casting technique the biomechanical properties of the polymer patch were assessed. This was done using the Biotester™ (CellScale, Ontario, Canada) biaxial tester, the detailed method is described in Section 3.2.3.

2.6.2 Instron Mechanical Assessment

The Instron Universal Testing was used to assess the effect of acid exposure on the polymer and the strength of adhesive bonds under varying conditions. The samples were loaded into the jaws of the apparatus and secured using sandpaper. Multiple repeats were performed as detailed in Section 5.3.

2.6.3 Effect of acid exposure on strength of polymer

POSS PCLU sheets 150µm thick were cast as described. Standard dumbbell shapes were cut using a flatbed laser cutter to allow tensile strength testing. These measured 20mm x 4mm 0.168mm+/-0.005mm. Seven samples were

placed in 0.1M HCl and incubated at 37ºC for one hour. The acid was removed, the samples were washed with PBS and placed in media and incubated at 37ºC. This was repeated twice a day for five days. Seven controls were incubated in standard media at 37ºC without the acid exposure. Tensile strength of the polymer was assessed using the Instron® Universal Testing System.

2.7 Surface Modification

Plasma surface modification of the POSS PCLU polymer patch was performed using oxygen plasma. This has been shown to increase wettability of biomaterials and improve cell adhesion[124, 151, 153].

Polymer discs were placed in the vacuum chamber of the plasma generator and exposed to oxygen plasma for varying times between 30 seconds and 4 minutes. The gas pressure was 0.4mBar. Samples were used in cell culture experiments within 24 hours of modification.

2.8 Surface Analysis

Surface analysis consisted of water contact angle measurement. Water contact angle gives a quantitative assessment of the hydrophobicity or hydrophillicity of a surface. The sessile drop technique was used with images recorded using a digital camera and water contact angle calculated using inbuilt software. Five samples of each polymer treatment condition were tested with all testing completed within an hour.

2.9 Statistical Analysis

Results were recorded in Microsoft® Excel® and statistical analysis performed using Graphpad Prism®. Statistical significance was taken at $p<0.05$. A minimum of three repeats was used for each set of tests where possible. Normality was tested and for parametric data group comparison testing was performed using ANOVA followed by post-hoc analysis (Dunnet) or T tests were used, whilst for non-parametric data the Kruskal-Wallis test was used.

3 Inflation Model

3.1 Introduction

A key aspect in developing an adhesive oesophageal patch is to create a robust model of the oesophagus to test repair methods including the burst pressure of a patch repair technique. Ex vivo models for testing oesophagus repair can range from simple test rigs designed to test one aspect of a proposed system such as burst pressure[193] or more complex bench top based systems looking at multiple mechanical properties[194, 195] and cellular responses[196]. In vivo models also exist in mice and the more physiologically and anatomically relevant pigs [197, 198]. The novelty of the approach used in this study is the simplicity of the model which can be easily replicated without the need for expensive and complex measurement, recording and analysis equipment. The ex vivo approach is also cheaper than using an in vivo live animal model and allows more experiments to be performed as a result.

The model needed for this project will attempt to recreate aspects of the oesophagus in form and function. The oesophagus normally exists in a collapsed state but can distend to allow the passage of larger boluses. The oesophagus can also be inflated with air to allow entry of instruments to facilitate diagnostic and therapeutic endoscopy including endoscopic mucosal resection, dilatation of strictures and placement of stents and feeding tubes.

3.1.1 Large animal in vivo models

Live porcine and other large animal models have been used extensively in biomedical research due to the size and physiological similarities to humans [199] however ethical, logistical and financial limitations make routine live animal porcine experiments challenging, as discussed by Gaarder et al. with reference to the use of live porcine models for training and research [200]. The porcine oesophagus is similar to the human oesophagus in anatomical location, sitting in the mediastinum, and structure, being composed of layers of striated and smooth muscle and associated connective tissue with a nerve plexus controlling peristalsis. The mechanical properties of the porcine oesophagus have not been compared systematically in the literature however

several studies have been conducted using porcine models of oesophageal disease[201] supporting the use of porcine tissue in the current study.

3.1.2 Small animal in vivo models

Small animal models are widely used in biomedical research and in the context of oesophageal tissue engineering, rabbit models have been used to test decellularised and hybrid polymer/cell scaffolds [202]. Whilst small animals are less costly in logistical and financial terms they are more difficult to operate on, have different mechanical properties and still present a significant ethical challenge [203]. Due to the size of the animal it is not usually possible to use the same equipment as is used clinically which may limit their use in testing specific commercial products.

3.1.3 Ex vivo models

Ex-vivo models have the advantage of using biological material and animal material in the form of whole organs and specific tissues can be readily obtained from commercial suppliers (Medmeat Supplies, Rochdale, United Kingdom). Ex vivo human tissue experiments are more challenging due to ethical issues relating to donor consent and safety concerns relating to transmissible infections but are widely used nonetheless particularly in the context of organ transplant research and surgical training. An advantage of ex vivo models of physiology is the ability to control more parameters limiting some of the variability seen on biological systems.

Ex vivo models cannot fully recreate the physiological environment they have been taken from and in most cases will not be fully functional, although models can be designed to provide physiological support to organs and tissues [198]. However in the setting of materials research human or animal tissues can undergo material property characterisation in the controlled laboratory setting with the oesophagus and blood vessels investigated in this manner [195, 204].

A number of researchers have used ex vivo oesophageal models for research and training; Yang et al. [195] developed a triaxial test rig for testing the effect of

diabetes on the biomechanical properties of the oesophagus using a rat model. Their system allowed the tubular oesophagus to be inflated with water, stretched and twisted, and the resulting deformity recorded using a video camera. Tanaka et al. developed a porcine model for training in oesophageal endoscopic mucosal resection[205]. The advantage of water inflation is that water is not compressible compared to air therefore smaller changes in volume can be reflected in the pressure changes. The model developed for this study is simplified to focus on burst pressure testing as the endpoint of the model so that different repair techniques can be compared.

3.1.4 Porcine Oesophageal Models

Porcine oesophagus is readily available and its similarity to human tissue is widely described in the literature[206, 207]. Porcine models are widely used in medical teaching due to similarities in tissue handling and anatomy[208]. Live porcine models have been developed to test endoscopic surgical techniques [209, 210] while conditions including traumatic injury, colorectal cancer, diabetes mellitus, short bowel syndrome and oesophageal disease have all been investigated using porcine models. In particular oesophageal conditions including gastro-oesophageal reflux disease[209] Barrett's Oesophagus and oesophageal cancer [201]have been investigated using porcine models. Ex vivo porcine models have been used for vascular burst pressure testing[211] and endoscopic training and non-destructive mechanical assessment models have also been evaluated[212].

3.1.5 In vitro cellular models

In vitro cellular models can be used to investigate aspects of cell behaviour and have been the mainstay of biomedical research for much of the 20th century. Protocols for in vitro experiments are well described in the literature and commercial development of equipment and consumables makes cell culture experiments cost effective (compared to in vivo models) across a range of biomedical research [213-215]. Cell culture experiments have been used extensively in the investigation of biomaterials to assess cell adhesion, toxicity and the interactions between materials and epithelial cells and fibroblasts[142].

Tan at al.[114] investigated the role of tubular polycaprolactone scaffolds in oesophageal replacement evaluating mechanical properties, crystallinity, and morphology as well cell interaction using murine fibroblasts. Hou et al.[115] looked at a novel polyester scaffold optimised for smooth muscle growth and orientation performing in vitro analysis of cell behaviour prior to implantation in a rabbit model.

3.1.6 Surgical Repair models

Animal models are widely used for assessing surgical techniques and technologies in tissue repair. Burst pressure testing is widely used in the assessment of anastomotic strength and enterotomy closure strength [193, 216, 217]. Live animal models allow testing of duration of repair techniques and long term effects on outcome[109, 218, 219]. There are, however, fewer ex vivo models used to measure the success or mechanical robustness of surgical procedures. Vanags et.al (2003)[194] used a combination of inflation and uniaxial stress loading to examine explanted human oesophageal tissue and Yang et al. (2006)[195] performed similar studies using rat oesophageal tissue. Ex vivo models, as previously explained, have several advantages in terms of quantitative measurement of mechanical properties or failure rates of repairs, in terms of cost but also in terms of reducing the animals used for in vivo testing (the oesophagus are obtained from pigs sacrificed for other experiments). The development of the model is therefore in line with the 3Rs (to replace, reduce and refine the use of animals for scientific purposes).

3.1.7 Aims

The aims of the experiments described in this chapter are:

1. To create a porcine oesophageal model to test perforation repair techniques.
2. To assess the reproducibility of the model between different oesophagus samples.
3. To measure the mechanical characteristics of the porcine oesophagus.
4. To test the burst pressure of different suture techniques.

3.2 Methods

3.2.1 Inflation testing

The aim of the inflation testing was to demonstrate that the model worked and that consistent results could be obtained within specimens and between different specimens.

Porcine oesophageal specimens were attached to the test rig and the inflated using water as described in detail in Chapter 2. The test was continued until some aspect of the model failed (e.g. the clip holding the end of the oesophagus came off or the specimen burst). Five to nine stills from each video, at increasing pressures, were analysed. Pressure was measured in kPa and the diameter of the oesophagus was measured in pixels and converted to a percentage increase in diameter. The diameter of the oesophagus was measured at each pressure point and stress strain curves were created. Preview© Version 8 for MacOS was used for the image analysis. Maximal pressures prior to failure were measured for each sample.

3.2.2 Burst testing

In order to test the burst pressure of the different repair strategies a 2cm longitudinal incision was made through the full thickness of the oesophagus. The oesophagus was then repaired using interrupted sutures, continuous sutures, 2 layers of sutures and glue-reinforced sutures. 3/0 PDS (poly p-dioxanone) was used as this is a standard choice of suture in surgery. For this set of experiments commercial cyanoacrylate adhesive was used to reinforce the sutured repair as it was readily available. Standard surgical suturing techniques were used. The tests were destructive and as such only three repeats of each experiment were performed to allow statistical analysis. The test was complete when water could be seen to be leaking from the repair demonstrating failure of a watertight seal.

3.2.3 Biaxial Testing

Biaxial testing of porcine oesophageal samples was performed to quantify the Youngs modulus in the axial and circumferential planes. The oesophagus was retrieved from recently terminated porcine specimens and tested within

four hours. Six 16mm x 16mm x 150μm samples were analysed using a biaxial testing system in a 37ºC water bath (Cellscale Biotester) (figure 3.1). Preconditioning, to ensure alignment of fibres, consisted of 5 repetitions of 10% load and equi-biaxial testing consisted of a single 40% load in the longitudinal and circumferential axes.

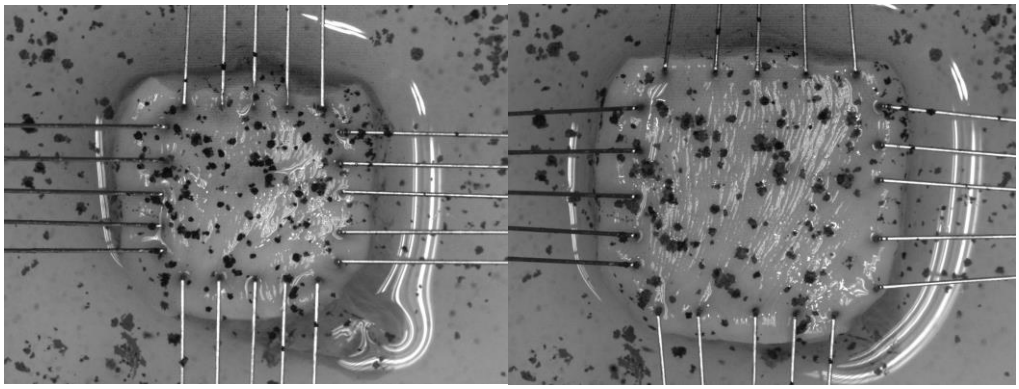


Figure 3.1: Mechanical testing of oesophageal sample loaded on CellScale biaxial testing system at 0% displacement (Left) and maximum displacement (Right).

3.3 Results

Mechanical testing of oesophagus tissue revealed the anisotropic nature of the tissue, with a stress of 4MPa causing a 40% displacement in the circumferential axis and a stress of 3MPa causing a 40% displacement in the longitudinal axis. The elastic modulus was 1.3kPa and 1.6kPa in the longitudinal and circumferential axes respectively (Fig 3.2) although this difference was not statistically different.

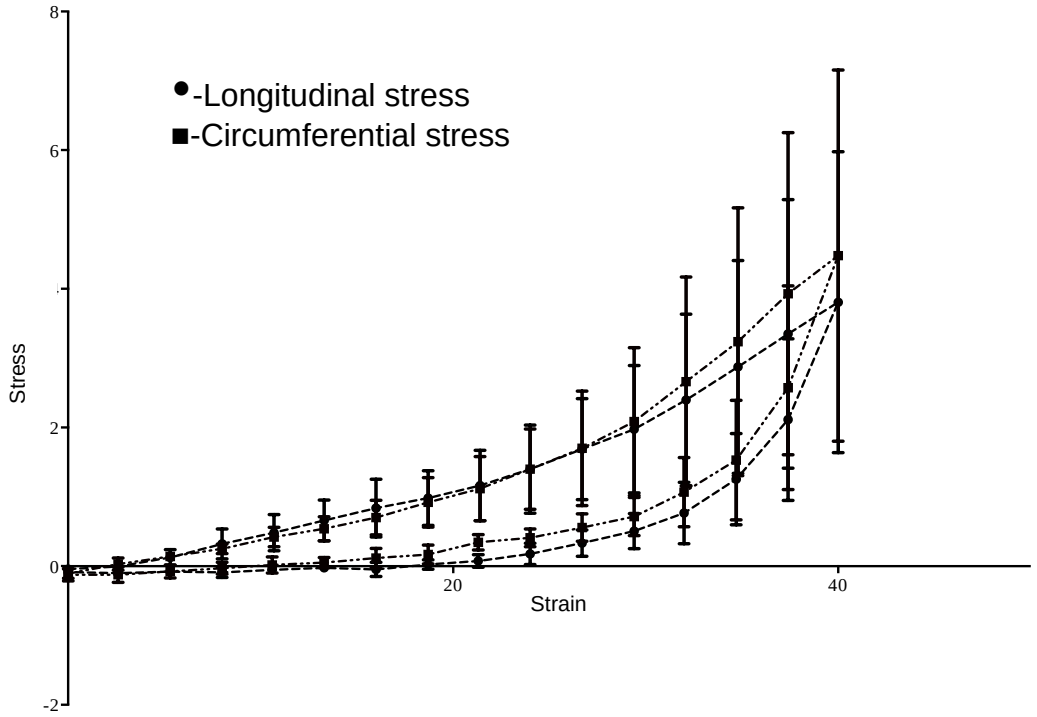


Figure 3.2: Stress (MPa) vs. strain (%) graph plotting longitudinal and circumferential axes of oesophageal patches.

The ex vivo oesophagus model created to test puncture repair was able to measure inflation up to a minimum of 69kPa and a maximum of 135kPa (Fig 3.3). The burst pressure average between different oesophagi was not significantly different (p=0.4) (Fig 3.3). The inflation results are presented as pressure required to increase the diameter of the oesophagus by 25%, 50% and 75% of the maximum pressure. Intra sample variance was observed with repeat inflations of the same oesophagus tissue (Fig 3.4a). Comparing the 3 runs (Fig 3.4a) showed no significant difference between the runs at each of the 3 inflation sizes (25%, 50%, 75%) and analysing the data comparing the inflation sizes to the runs also showed no significant difference (Fig 3.4b). The absence of correlation between order of inflation and pressure required to inflate to a specific size suggests any damage to the oesophagus tissue following repeated inflation of the oesophagus did not effect the overall material properties as assessed in this model. This finding would allow for repeated use of samples within different experiments.

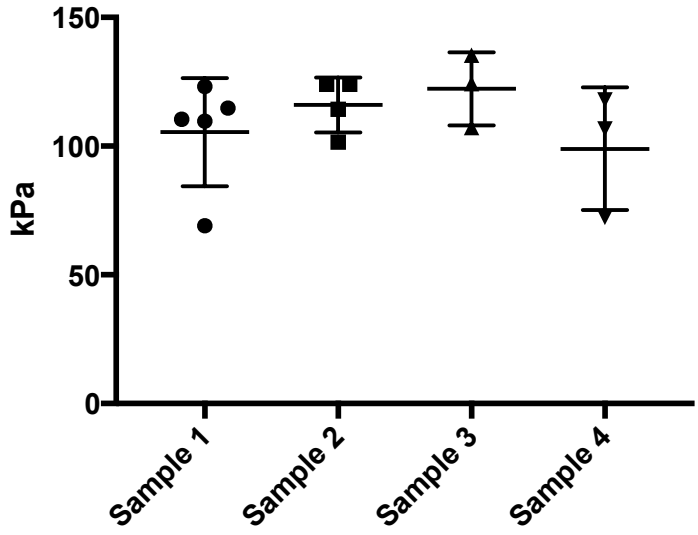


Figure 3.3: Variability in maximum pressure achieved within the inflation model. Four samples tested, no significant difference between maximum pressures in samples. The number of repeats did not appear to affect the results.

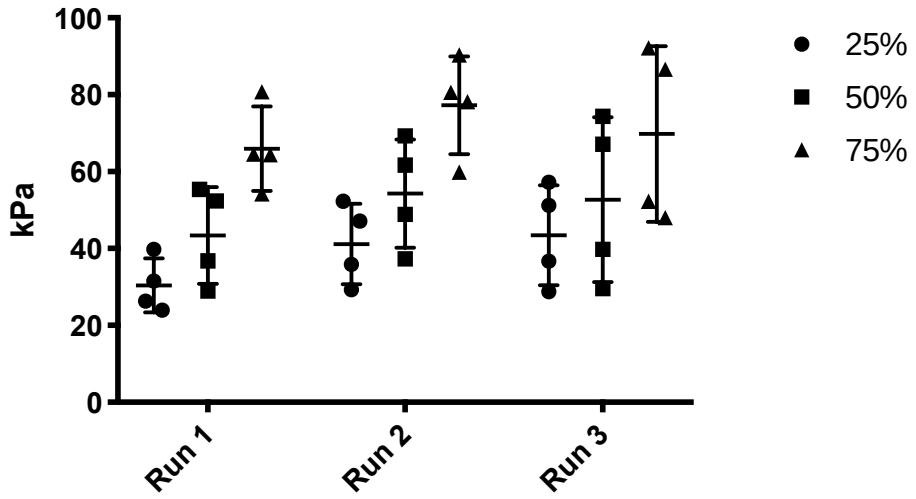


Figure 3.4: Variability between each inflation run at 25%, 50% and 75% of maximum inflation. There is no significant difference between each run implying consistency of the model.

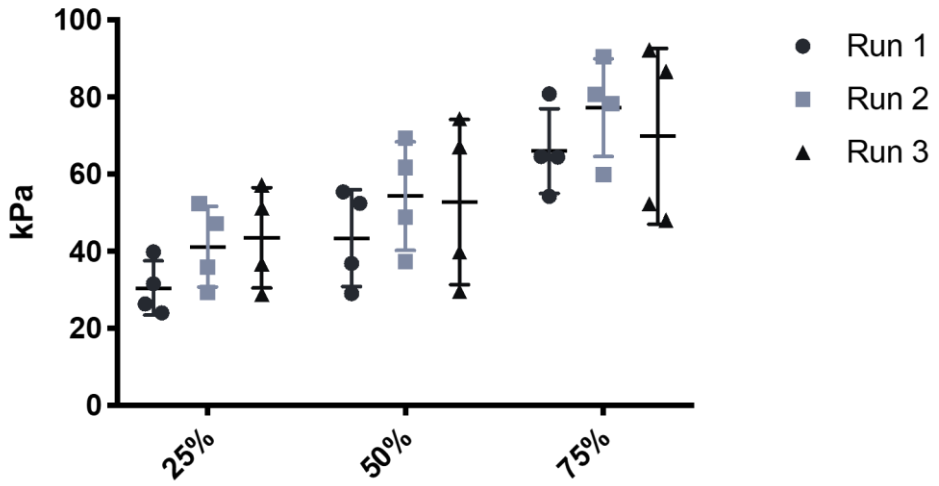


Figure 3.4b: The variability between runs (repeats) for each level of inflation. There is no significant between runs (repeated inflation of the same tissue).

The ability of the inflation model to assess the burst pressure of a repair was assessed using a continuous suture closure technique. The results show the majority of points falling within 1SD of the mean (Figure 3.5).

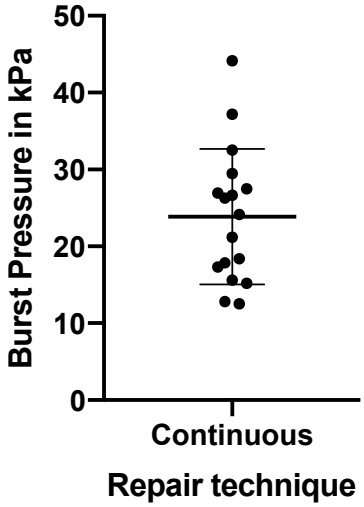


Figure 3.5: Validation of model for testing repair technique. Continuous suture. N=17, mean failure pressure 24kPa, SD 8.8kPa.

3.4 Discussion

This stage of the study has demonstrated the utility of a simple ex vivo bench top model for testing oesophageal repair techniques. Whist ex-vivo models have been developed for gastrointestinal repairs[193 220 221], this is the first report of an ex vivo bench top model to test oesophagus perforation repair. The model was low cost and relied on a relatively low level of technical

expertise although supply of material was a limiting factor at times so the number of repeats was low in some experiments. It has demonstrated the level of repeatability both within samples with repeated inflation and between samples from different pigs as demonstrated by the maximal burst pressure tests (Fig 3.3) and demonstrated that the burst pressure of repair techniques can be assessed (Fig 3.5). The reliability of the model and the ease of use make the model an attractive prospect for future experimental work and could be cross-purposed for training use as well.

One challenge with using animal tissue is the variability that occurs. This was evident when looking at the results of the inflation model and the stress-strain curves generated. Whilst within each sample the variability was minimised between samples there was variation between samples in the inflation pattern. Whilst all samples were taken from pigs bred commercially for medical training applications of a similar age and size, the same breed and raised in standard conditions, there was wide variation in the measurement of the mechanical properties. Additionally the time from retrieval of the oesophagus to testing and the handling after retrieval may affect the results. Early work in the project used fresh porcine oesophagus from animals being used for other experimental work and this fresh tissue was chilled and used within 24 hours. The supply of this material was limited and for the purposes of the main series of experiments fresh frozen tissue was used. Freezing will have an effect on the material properties however within the financial and ethical constraints of this work it was not possible to fully assess this. All the experimental porcine specimens were handled in the same way to reduce inter-sample variance as much as possible. Frozen samples were defrosted overnight in a fridge and used in experiments immediately. All of these factors can cause problems when experiments rely on reliable models that have a predictable response. This may also reflect the inherent in vivo variability and reliability of animal studies as well. Maximum burst pressure was, however, a repeatable measure and within the context of this study was a useful feature of the model.

Techniques for limiting variation including controlling initial experimental parameters and increasing the number of repetitions of each experiment. In the context of tissue experiments the number of repetitions is limited by access to the tissue and as well financial considerations. Parameters that could be controlled in this study included the size of the incision in the oesophagus and the way the oesophagus was loaded within the test rig and this variation was minimised by having a single person perform all the experiments, aiming to complete each experiment in a single session so that within each run of experiments environmental factors are similar. Similarly variation in the size of the linear incision made in the oesophagus was limited by having a standard length and each incision made by the same person.

Variation in the analysis of the inflation images was an important consideration. The diameter of the oesophagus in each image was measured by drawing a line from one edge of the oesophagus to the other. Image analysis software recorded the line length. There is obviously the potential for variation in the placement of the line which would affect the diameter. Strategies to limit this element of variation include multiple measurements within each image or having more than one person take the measurements and an average of the measurements taken. Within the limitations of this study the measurements were all taken by the same person and the location of the cursor points that created the line to be measured was consistent in each sample as far as possible. By analysing multiple images from each inflation run variation was reduced but not eliminated.

Various approaches for burst testing of hollow organs have been reported. Intraluminal infusion of dye solution has been used to test novel closure techniques[193], radiological contrast has been used to measure leakage[220] and in clinical practice gas insufflation is used[221]. Initially air insufflation using an endoscopic inflation device designed for inflating balloons was explored in the ex vivo model design. This approach, however, failed due to the volumes of air needed to be inflated and the relatively low sensitivity of the pressure gauge as described in Chapter 2. The use of a water pump and a digital pressure gauge allowed much more control within the system and gave more

reliable readings of pressure as demonstrated in figure 2.2. This approach has been previously reported for rat oesophageal tissue[222] and human oesophageal tissue[195].

Testing the repair technique attempted to validate the utility of the model. There was a wide variation in the burst pressures achieved with the continuous suture technique but the majority of the readings fell within 1 SD of the mean suggesting the model is reliable in this context. As discussed above variability is a challenge when dealing with biological tissues however this experiment does demonstrate the ability of the model to test initial water tightness of the repair which is a key feature in the development of the oesophageal repair patch.

This model has advantages over more sophisticated models, the porcine tissue is cheap and readily available, the tissue is recognised as being an acceptable model of human tissue and the test rig is a easily constructed without the use of specialist equipment. This makes it a highly relevant in the application of teaching, practical skills can be quickly and accurately assessed, and in the initial development of novel technologies where investment in advanced testing cannot yet be justified. The model uses a pulsatile continuous flow pump which has the effect of increasing the pressure in a continuous manner. The model could be improved by using a pump that is able to provide a fixed pressure to the system that could be released without the having to push the model to failure. This would allow, for instance, pressure testing of repair techniques without having to disrupt the repair in the process.

Having established a reliable model allowing pressure testing of the oesophagus the next stage of the study is to develop the patch that will be used to repair oesophageal the oesophageal perforation.

4 Patch Development

4.1 Introduction

The aim of this project is to create a patch that can be used to seal defects in the oesophagus; these defects may be due to iatrogenic injuries sustained during endoscopic procedures, spontaneous perforations, post operative leaks or traumatic injuries ranging in size from a pinhole leak to a tear measuring two to three centimetres. This patch should stick to mucosa or underlying tissue, and support normal oesophageal function while promoting functional tissue regeneration. As described in previous chapters materials used in tissue regeneration settings can be biological or synthetic and must support the function of the physiological system. The polymer patch should have mechanical properties that match the mechanical properties of the oesophagus including strength and stiffness. The surface chemistry of the patch should optimise protein adhesion to allow cell adhesion as well as optimising the effect of the tissue attachment. Altering the wettability of the surface can vary surface chemistry that will affect protein adhesion and cellular interactions. For this project the synthetic polymer POSS PCLU is being used.

POSS PCLU is a propriety polymer consisting of polyhedral oligomeric silsesquioxane (POSS) nanoparticles suspended in poly(e-caprolactone urea) (PCLU) and was developed as a degradable evolution of a similar POSS containing polyurethane POSS PCU (UCL-Nano™)[134]. Its properties and applications have been extensively investigated as part of previous PhD work in this laboratory[105, 135-137]. POSS PCU has been used for clinical applications include tracheal replacement[223] and tear duct replacement[138] while vascular grafts and synthetic scaffolds for tissue regeneration have been investigated in vitro[137, 139, 140]. POSS PCLU has been studied in the context of skin tissue engineering, vascular graft and paediatric applications[141, 142].

POSS PCLU has been proposed for a number of biological applications due to its tunable physical properties, slow degradation rate compared to

polyglycolic acid (PGA) or polylactic acid (PLA) and promising biological interactions[105]. POSS PCLU has been shown to degrade in time periods of weeks to months while over shorter time periods (days to weeks) no significant degradation occurs[224].

POSS PCLU was chosen as a suitable polymer in this study as a degradable polymer with tunable material characteristics by virtue of the POSS content that would allow the patch material to be modified to both match the mechanical properties of the oesophagus and optimise function while supporting tissue regeneration.

This chapter will focus on the development of the POSS PCLU patch, assessment of stiffness, assessment of wettability, surface chemistry modification in the form of plasma surface modification and cell-material interactions.

4.2 Methods

4.2.1 Casting technique

POSS PCLU sheets were cast as described in Chapter 2. The thickness of the sheets was important as this would dictate some of the bulk properties of the polymer patches including substrate stiffness. During the development of the patch it became obvious that below approximately 75µm thickness the resulting polymer sheet was too fragile to handle comfortably behaving like culinary food wrap, sticking to itself and tearing very easily. Polymer sheets 100µm and 150µm thick were cast for this series of experiments using the formula detailed in chapter 2. Cast sheets of polymer were cut into patches 20mm by 50mm and the thickness measured using digital callipers. 19mm circles of polymer were cut out using a laser cutter or die stamp for cell culture experiments. 10mm by 20mm strips were cut for surface chemistry assessment.

4.2.2 Bulk Material Properties

Having established a robust and reliable casting technique the biomechanical properties of the polymer patch were assessed. This was done using the

Biotester™ (CellScale, Ontario, Canada) biaxial tester and the Instron uniaxial testing system. PCLU sheets 130µm thick were cut into five15mm by 15mm squares. Each square was tested on the biaxial testing system (Cellscale biotester). Preconditioning consisted of 5 repetitions of 10% load followed by a single 40% load in X- and Y-axes.

4.2.3 Effect of Acid Exposure

Symptomatic gastro-oesophageal reflux is common in the UK effecting approximately 10% of the adult population[225] however patients undergoing endoscopic mucosal resection, a recognised risk for perforation are likely to have higher rates of reflux due to the underlying pathophysiology[226]. The lower oesophagus is exposed to highly acidic conditions due to intermittent gastro-oesophageal reflux which is associated with the development of intestinal metaplasia, high grade dysplasia and invasive cancer. Materials used in tissue regeneration in the oesophagus will need to withstand these acidic conditions in the timeframe they are functional. POSS PCLU samples were exposed to acid conditions in a model mimicking the human lower oesophagus and compared to samples stored in cell culture media following the method described in chapter 2. Bulk properties of the polymer samples were assessed.

4.2.4 Material-Cell Interaction

As discussed previously biocompatibility is a concept that can be difficult to define as tissue-material interactions are system and situation specific. In the development of an oesophageal patch the biological system is the damaged oesophagus consisting of amongst others epithelial cells, inflammatory cells and fibroblasts that, along with the extra cellular matrix (ECM), make up the oesophageal tissue.

In this series of experiments the effect on cell behaviour of the polymer patch was assessed. Two different thicknesses of patch were used to determine the effect of substrate stiffness on cell attachment and behaviour; POSS PCLU samples were compared with tissue culture plastic acting as a control.

Detailed experimental technique is described in chapter 2. Briefly three 24 well plates were set up for testing at 24 hours, 72 hours and 96 hours. The media was changed 24 hours prior to running the assay. The metabolic activity was assessed at each time point using alamarBlue and the results were normalised to cell number using a Total DNA assay.

4.2.5 Polymer surface Modification

Surface chemistry effects cell attachment and subsequent behaviour [152, 227, 228]. Surface chemistry can be altered using a variety of techniques described in previous chapters. For this study plasma surface modification was used to alter the hydrophilicity of the polymer surface. Increasing the hydrophilicity has been shown to enhance tissue integration and angiogenesis[151]. The plasma surface modification process is described in detail in the Chapter 2. The POSS PCLU samples were treated with oxygen plasma for varying times from 30 seconds to 4 minutes.

The effect of the plasma treatment on the surface chemistry was assessed by measuring the Water Contact Angle using the sessile drop technique. An unmodified sample of POSS PCLU acted as a control. Each sample was measured five times a process which took approximately 5 minutes. The tests were carried out immediately after the treatment. The effect on cell attachment and behaviour was assessed using the same cell culture protocols described previously. The duration of effect and the effect of storage conditions was assessed by storing treated polymer samples in ambient conditions or in a vacuum chamber. Water contact angle was measured after one week.

4.3 Results

4.3.1 Casting technique validity

The casting technique was shown to be reliable for the desired thickness and the variability was minimised. 150□m sheets measured 151□m +/- 2□m and 100□m sheets 94 +/-3□m□

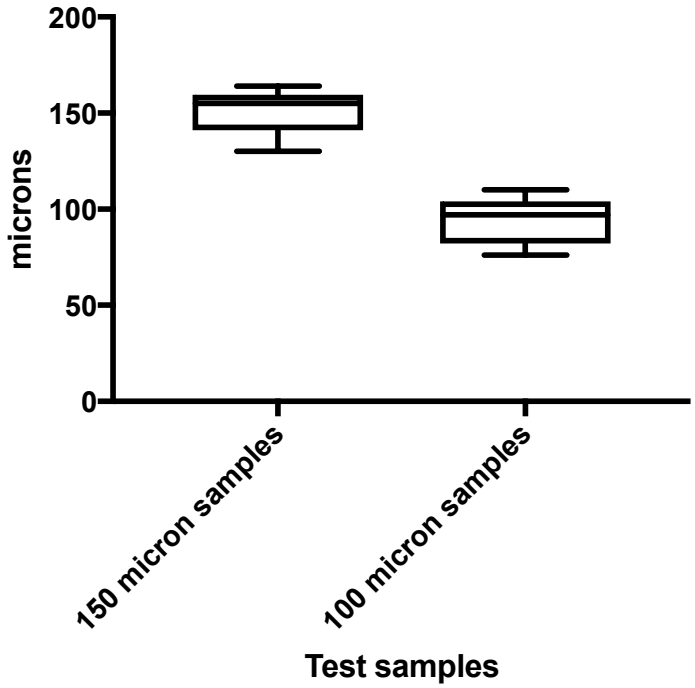


Figure 4.1: Comparison of two casted sheets cut into patches. Difference is significant (P<0.0001 Unpaired T test). N=21 for □□□□m and N=19 for □□□□m sheets.

4.3.2 Mechanical Properties

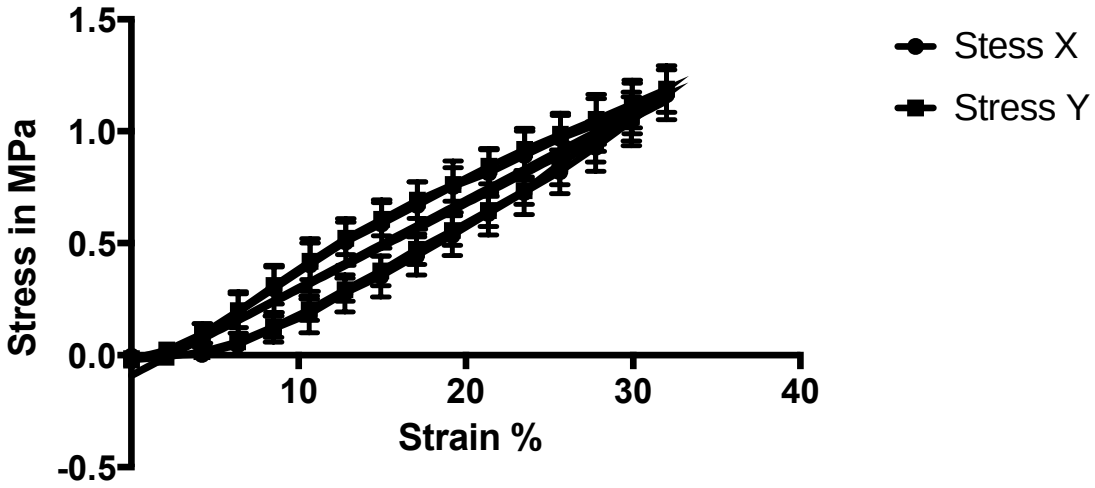


Figure 4.2: Stress strain graph produced from biaxial mechanical assessment of POSS PCLU patch, patch thickness 130□m. N=5.

4.3.3 Acid Exposure

Incubation of the POSS PCLU in acidic conditions for 1 week had no significant effect on the breaking strain of the polymer.

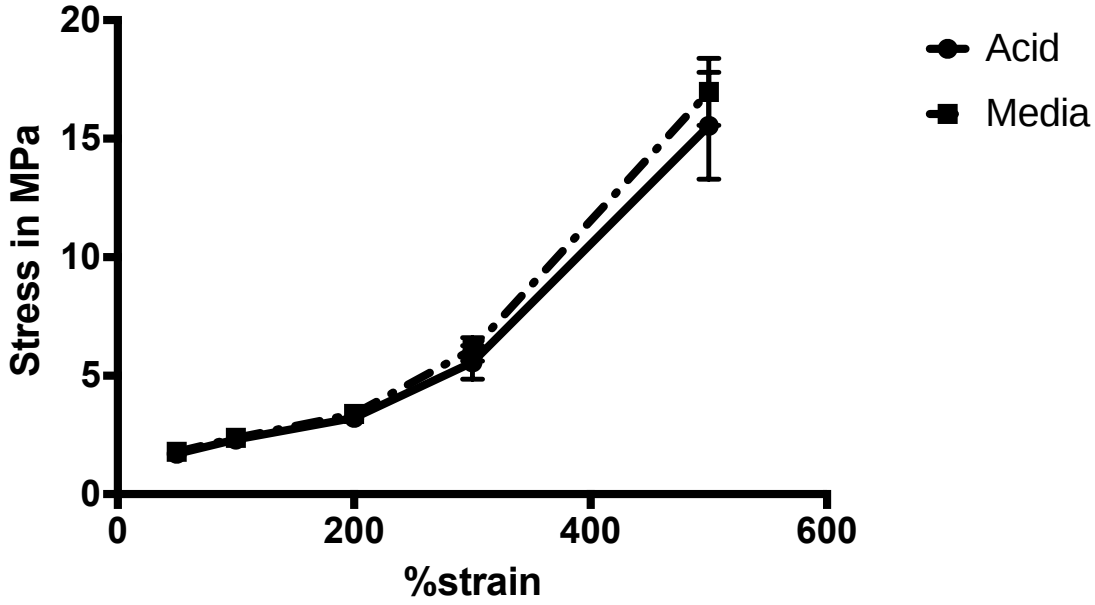


Figure 4.3: Effect of acid exposure on POSS PCLU. N=7 for each condition. No significant difference between the two samples.

4.3.3 Patch thickness effect on cell growth

The effect of the thickness of the patch on cell growth was assessed using cell culture techniques described in Chapter 3. Metabolic activity at 24 hours typically represents cell attachment. There was no significant difference in human dermal fibroblast (HDF) attachment between 100µm patches and 150 µm patches. HDF demonstrated more attachment on the tissue culture plastic control than either patch thickness.

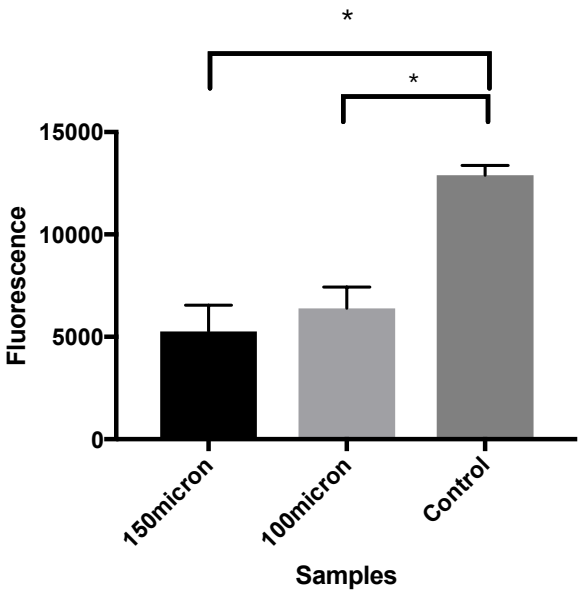


Figure 4.4: Effect of patch thickness on HDF attachment. Attachment of cells on 150µm samples, 100µm samples and TCP control after 24 hours. *P<0.0001, unpaired T test, N=6.

At 72 hours metabolic activity had increased in all samples however activity on the 100µm sample has matched the control tissue culture plastic.

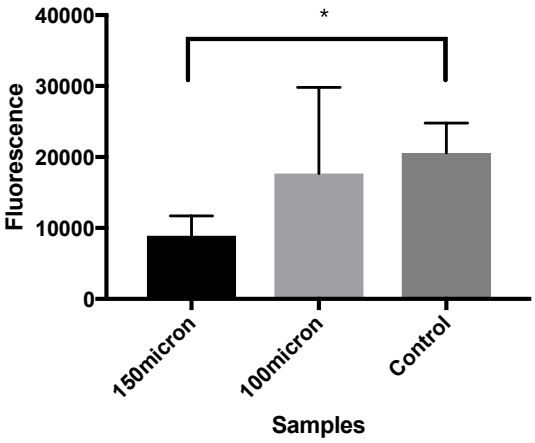
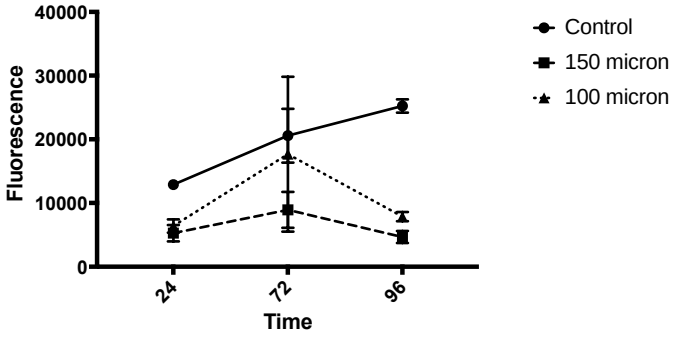
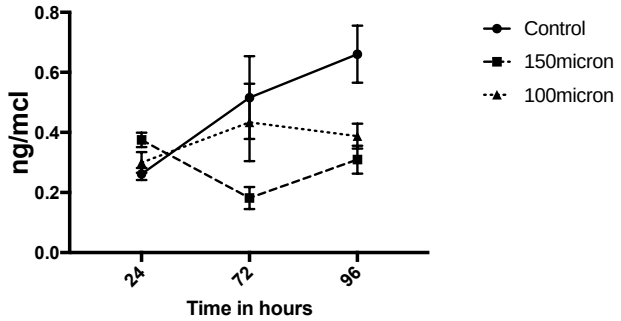


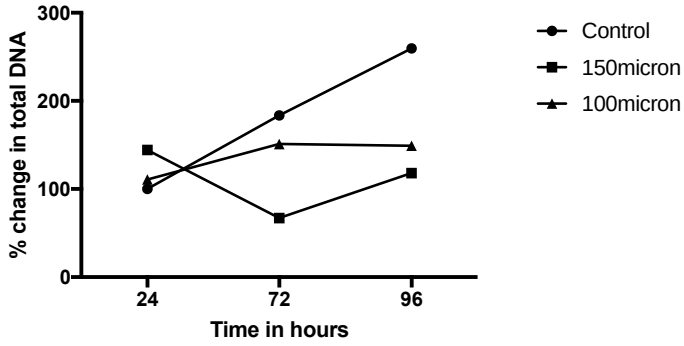
Figure 4.5: Metabolic activity of cells on □□□□m samples, □□□□m samples and TCP control after 72 hours. *P=0.0002, unpaired T test, N=6.



a: Metabolic activity of HDF over 7 days in TCP control



b: Amount of DNA in each sample over time



c: Percentage change on DNA concentration

Figure 4.6: Cell behaviour comparing □□□□m samples and 100□m samples, control is tissue culture plastic. N=6 for each condition.

4.4.3 Effects of plasma surface Modification

O_2 plasma treatment resulted in a reduced contact angle. The contact angle for untreated POSS PCLU was significantly higher than for any of the treated samples ($P<0.001$). The duration of treatment had no effect on the water contact angle.

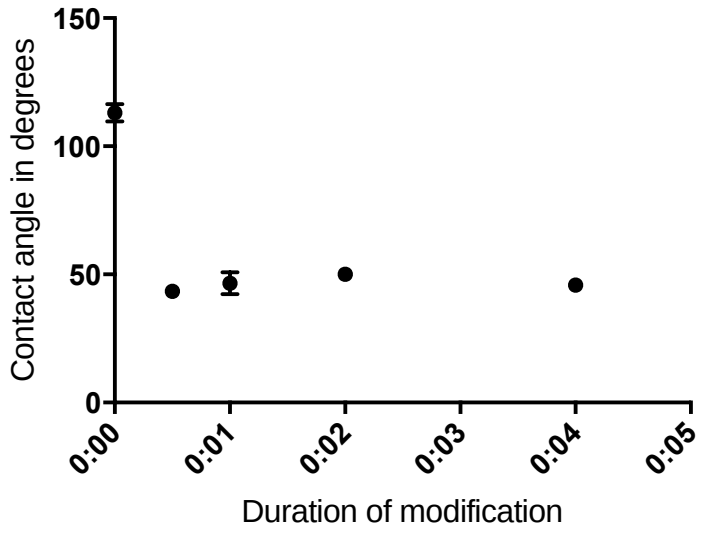


Figure 4.7: Duration of plasma treatment and effect on contact angle. Five repeats for each time test sample. There was a significant decrease in contact angle between treated and untreated samples ($P<0.0001$ one way ANOVA). No significant difference between the duration of plasma treatment was observed.

Storing the samples in a vacuum appeared to preserve the effect of the plasma treatment compared to storage on ambient conditions. This is important when considering both clinical applications and also experimental work when considering timing of experiments and the preparation of materials.

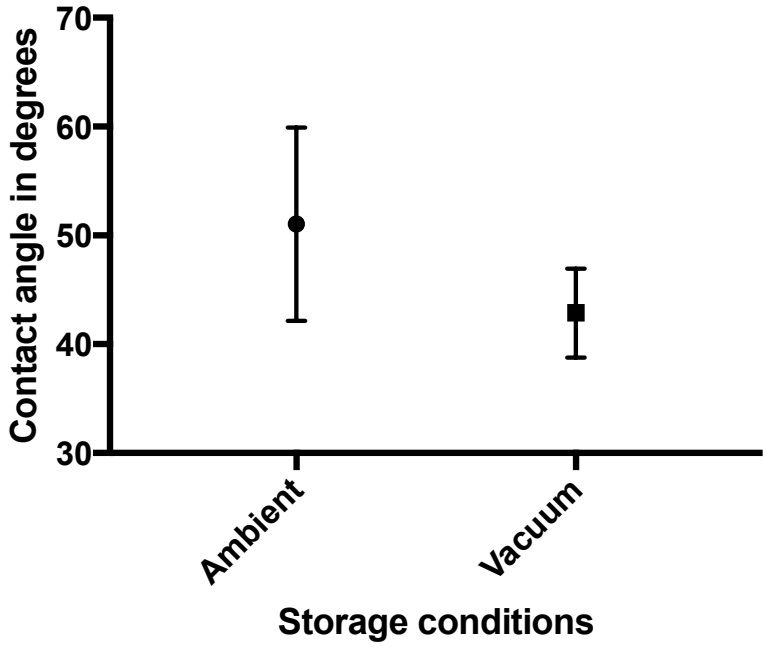
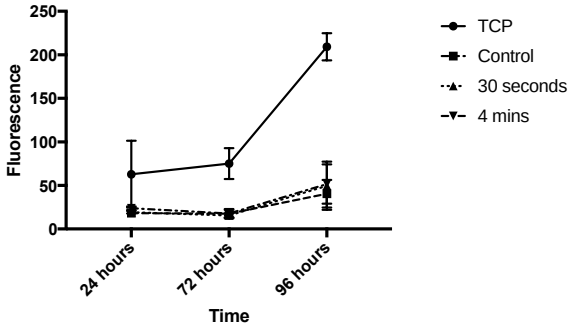


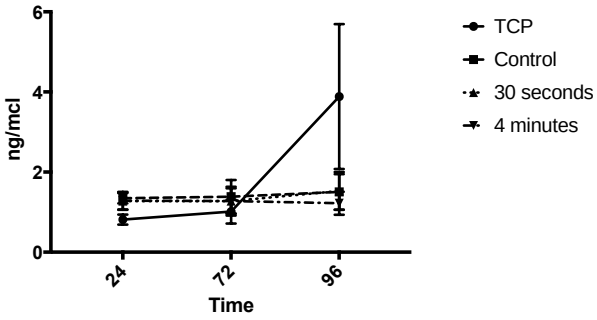
Figure 4.8: Plasma modified polymer patch samples were stored for one week in ambient conditions and in a vacuum flask, N=21. Water contact angle was significantly greater in the ambient storage conditions P=0.0002 unpaired t test.

Surface modification appeared to have little effect on the behaviour of cells growing on POSS PCLU with similar growth observed on treated and untreated polymer samples. Cells were significantly more active on the tissue culture plastic at all time points.

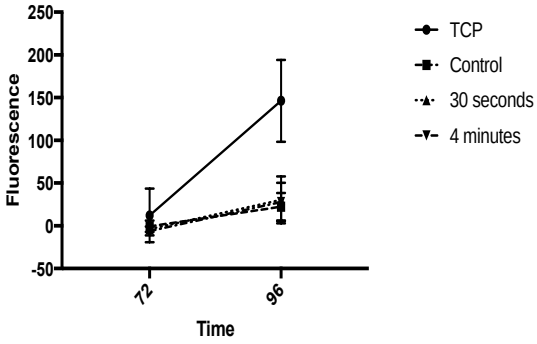
There was markedly reduced metabolic activity and cell number in all the POSS PCLU samples when compared to the tissue culture plastic control.



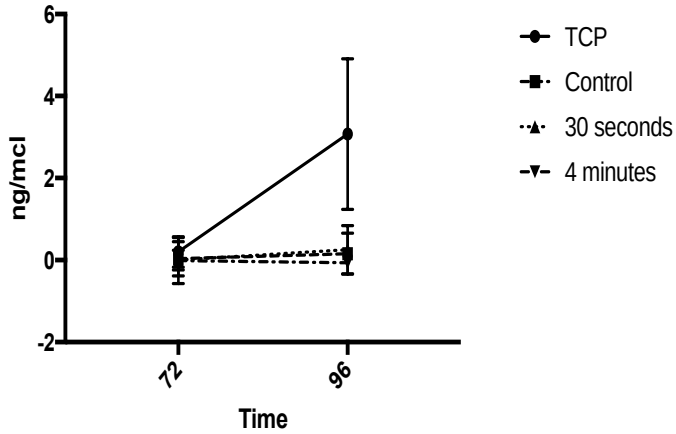
a: metabolic activity of cells on modified polymer samples



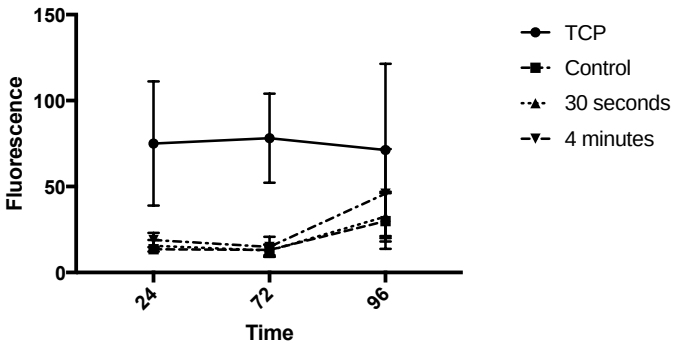
b: total DNA at each time point on the treated and untreated polymer samples and control



b: metabolic activity normalised to 24 hours



c: amount of DNA normalised to 24 hours



d: metabolic activity per unit of DNA

Figure 4.9: Metabolic activity and total DNA of cells at 24 hours, 72 hours and 96 hours for Tissue Culture Plastic (TCP), unmodified POSS PCLU, 30 seconds of treatment and four minutes of treatment. Five repeats for each condition. Results are expressed in concentration of DNA and fluorescence.

4.4.4 Discussion

The most important findings from this set of experiments are that there is no difference in cell attachment or behaviour based on the thickness of the material or the surface chemistry modification. Over the course of a week there is no effect on the bulk material properties of physiological exposure to acidic conditions. The surface chemistry changes caused by the plasma modification fade when the material is stored in ambient conditions compared to under vacuum.

Biaxial assessment of the polymer showed a maximum stress of 1.3MPa at a strain of 32%, this compares to a stress of 4MPa at a strain of 40% for porcine oesophagus. As expected the variation in the polymer is much less than in the porcine tissue. A significant challenge when using biological tissue in this type of experiment is the variability and typically high numbers of repeats are needed to reduce the variability to gain statistically meaningful data. This was outside the resources of this study. In healthy human volunteers pressures within the oesophagus range from 7kPA to 15kPa[229] with rupture pressures conducted in explanted specimens ranging from 32 to 55kPa[194].

This series of experiments intended to examine a range of properties of the POSS PCLU polymer patch. The wettability assessment clearly demonstrates that oxygen plasma treatment increases the hydrophilicity of POSS PCLU

consistent with work looking at POSS PCU and PCL[151, 153]. The duration of treatment does not seem to affect the degree of change which is in line with work looking at polypropylene[230]. This was further assessed by looking at the durability of the modification and the effect of environmental conditions.

The effect of storage conditions on the surface chemistry was clear and is an important consideration when considering commercial exploitation of the concept of a polymer patch. This project has focused on a degradable polymer on the basis that the clinical application only requires the patch to be in place for the duration of the healing process. In a real world situation the patch may be stored for months or years before being used and storage conditions are clearly important.

The cell culture experiment demonstrates that there appears to be little effect of the surface modification on the metabolic activity of the cells when comparing modified and unmodified POSS PCLU however the activity is markedly lower compared to cells grown without POSS PCLU this varies from other studies[149] using oxygen plasma. A significant problem in oesophageal tissue engineering is fibrosis and stricture formation following damage to the epithelial lining of the oesophagus. The apparent reduction in cell growth associated with the presence of POSS PLCU may be beneficial in this application.

In subsequent work this could be examined using an in vivo animal model and assessing the degree of fibrosis generated by POSS PCLU. Yilidirimer et al.[105] undertook similar work but did not look specifically at the oesophagus.

The oesophagus can be exposed to acidic conditions in the both normal and pathological states. The patch was shown to be resistant to the degrading effects of acid over the course of a week which matches the findings of Gu et al.[224]. It is worth noting that in the clinical scenario where this patch might be used it is likely the patient would be on proton pump inhibitors that would substantially reduce the amount of acid produced in the stomach so limiting the exposure to the oesophagus to low pH conditions.

When the polymer is treated with oxygen plasma there is a significant increase in wettability but this appears to have no effect on cell behaviour. Increasing wettability increases protein adhesion which can increase cell adhesion[149] although this is not seen in this study. In this study the behaviour of cells cultured on POSS PCLU is different to cells cultured on tissue culture plastic which may represent an effect of the sterilisation process which was the same for all the POSS PCLU patches but was not applied to the tissue culture plastic which was sterilised using commercial gamma radiation sterilisation and used straight from the commercial packaging.

Increasing the wettability of the polymer does not seem to have had any effect on the cell attachment or behaviour it may be that there are other effects that modifying the surface chemistry has that have not been examined in this study. In particular the morphology of the cells adherent to the polymer could be assessed and has been shown to vary based on surface chemistry differences [149]. In this study oxygen plasma was used to treat the polymer surface however other molecules and functional groups including Helium[149], allylamine groups [154], growth factors[231] and peptides[232] aiming to functionalise polymers and biomaterial implants.

5 Adhesion Model

5.1 Introduction

For this study commercially available fibrin glue has been chosen due to the low toxicity, purity and reliability of supply and extensive favourable literature regarding clinical use.

5.2 Methods

Instron uniaxial testing protocol is described in chapter 2. Surface modification has been discussed in Chapters 2 and 4. Experimental protocol for effect of exposure to media on fibrin attachment is described in Chapter 2. POSS PCLU patches, as used on previous experiments in this study, were used and porcine oesophagus was used as for the tissue attachment experiments. Polymer patches 20mm by 50mm were attached to similarly sized sections of oesophagus with a 20mm overlap. These were secured in the Instron jaws using sandpaper to ensure a tight grip.

5.3 Results

5.3.1 Strength of fibrin glue between polymer and porcine tissue

There was no difference in the strength of the attachment between polymer and epithelium and polymer and submucosa. Fibrin glue strength between polymer and polymer was significantly stronger (Figure 5.1).

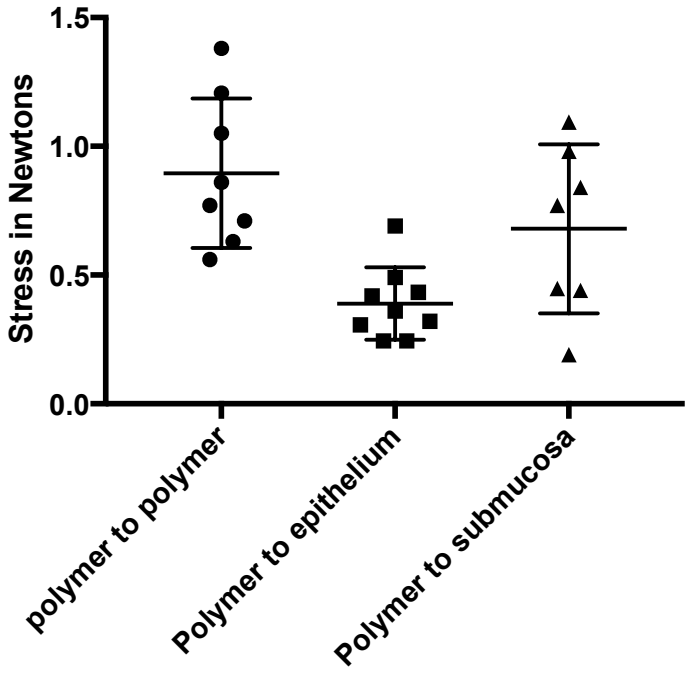
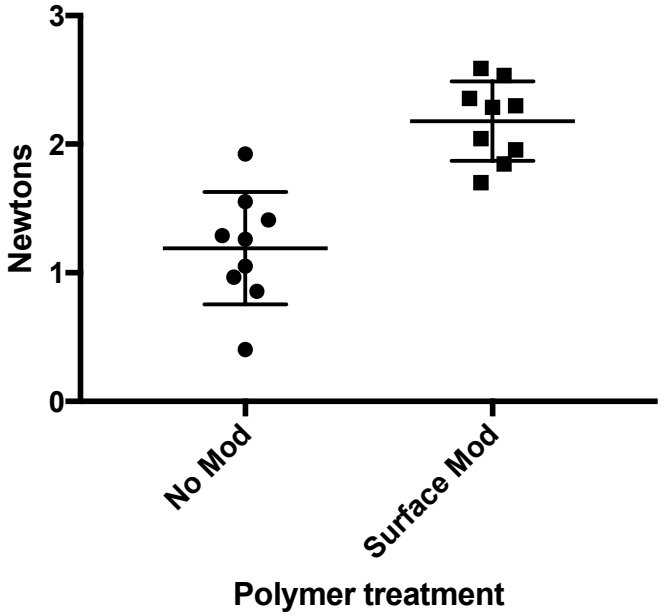


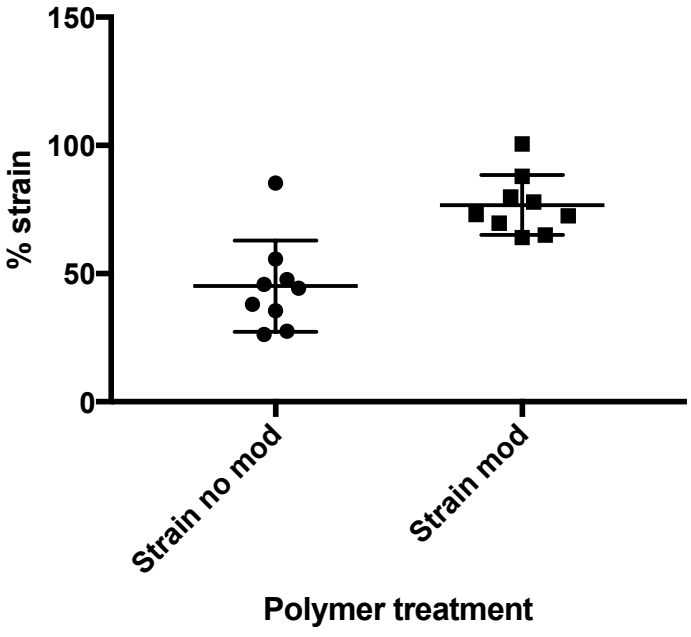
Figure 5.1: Mean and standard deviation for uniaxial stress testing of adhesive strength of fibrin glue. One-way ANOVA p=0.0022. Polymer to polymer N=8, polymer to epithelium N=9, polymer to sub-mucosa N=7.

5.3.2 Effect of Surface Modification on polymer to polymer fibrin adhesion

Polymer patches were subjected to plasma surface modification as described in Chapter 2 and Chapter 4. The strength of fibrin attachment was significantly greater following surface modification.



a:



b:

Figure 5.2: Effect of plasma surface modification on fibrin adhesion strength. Statistical comparison performed using an Unpaired t-test, N=9. a: Strength in Newtons $p<0.0001$ b: %strain $p=0.004$.

5.3.3 Effect on fibrin glue strength after exposure to media

The samples left in media for 1 week demonstrated significantly greater adhesive strength compared with samples tested after 30 minutes although the variability is increased as can be seen in figure 5.3.

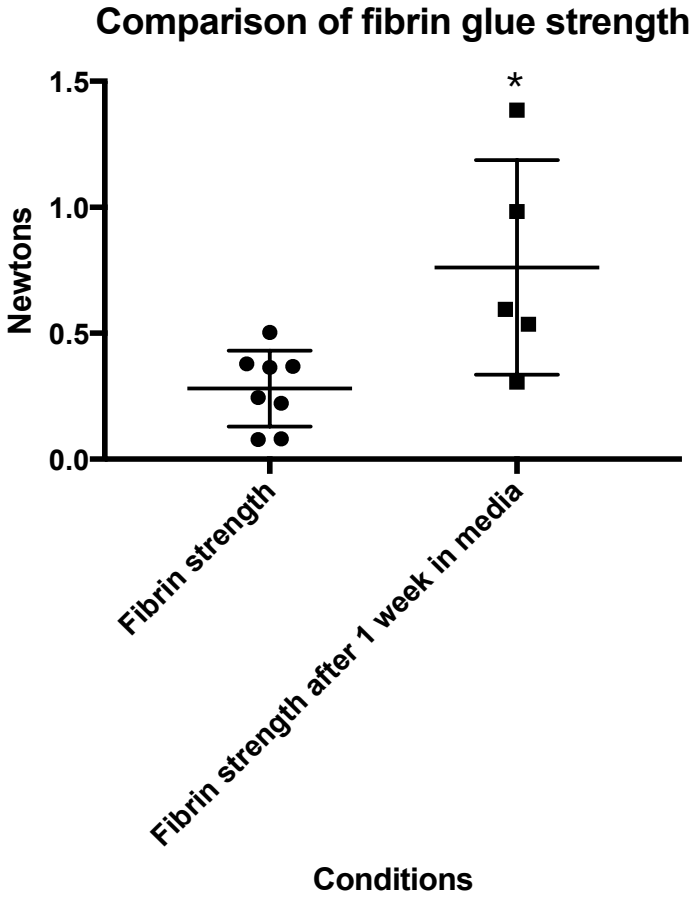


Figure 5.3: Force in Newtons required to disrupt the adhesive in the immediate samples and the samples incubated for one week. P=0.0186. N=5 for media storage and N=8 for immediate testing. Mann-Whitney statistical test was used for this non-parametric data.

5.4 Discussion

These results support the potential role of fibrin glue in the context of sticking a polymer patch to the lining of the oesophagus. The initial experiments looking at polymer to oesophagus aimed to model the effect of a straightforward perforation where the oesophagus maintains the majority of the endothelial lining and perforation following endoscopic mucosal resection where an area of endothelium will have been removed.

The variability in the results is likely due to a combination of factors. A significant contribution will be the uneven distribution of fibrin on the two adhesive surfaces. The commercial fibrin glue used in this study consisted of a double-barrelled syringe containing the soluble fibrinogen and other ingredients as described above. When the plungers are depressed the solutions mix in the nozzle and polymerise rapidly. This means that the glue must be applied quickly to allow even distribution before the polymerisation reaction is too advanced. With practice this could be achieved.

The clinical setting of a polymer patch secured with fibrin glue for the management of oesophageal perforations would see the patch being in place for an extended period of time. The fibrin glue adhesive strength increased following a week of incubation on media although as can be seen from the figure the variability is increased. This likely due to variation in the application of the glue and the extent to which media has seeped between the attached surfaces thus weakening the bond. It is reassuring that within this experiment even the weakest attachment is as strong as the mean attachment immediately after application.

As described in the introduction fibrin attaches to cells via the RGD moieties on cell surface integrins. Attachment to the polymer appears to be enhanced by altering the surface chemistry making it more hydrophilic. The strength of the attachment is doubled following plasma modification while the strain to failure is increased by approximately 50%. This straightforward technique for altering surface chemistry may prove beneficial in the clinical application of this technique.

6 Discussion

6.1 Project Outcomes

The aim of this project was to investigate the initial development of a polymer patch for the treatment of oesophageal leaks and perforations. An ex vivo model of oesophageal inflation was created to allow assessment of the material properties of the oesophagus progressing to testing repair techniques. This set of experiments demonstrated the utility of the model. The polymer patch was created from POSS PCLU, a proprietary degradable polymer with tunable material properties that has been used in a variety of preclinical applications. The polymer was reliably cast and easy to handle while being resistant to physiological acidic conditions in the lower oesophagus. The polymer surface could be modified using cold plasma to increase wettability. This alteration of surface chemistry did not change cell adhesion or behaviour however increasing wettability did increase the strength of fibrin adhesion. In addition to fibrin glue strength increasing due to increased wettability, the strength increased over time. In summary this project has demonstrated the utility of a bench top ex vivo model of the oesophagus in testing oesophageal repair and demonstrated the feasibility of creating a polymer patch that can be attached to the oesophagus using a biological adhesive.

6.2 Model

Creating models of biological processes is an established technique to allow experiments to be performed that facilitate close control of specific elements of the biological system in question. No model will recreate the system perfectly but models can be developed that allow specific questions to be answered. In vitro models can be used to investigate cellular processes and mechanisms while animal models can recreate complex biological systems to test pharmaceuticals, surgical implants, novel surgical techniques or to develop and test new procedures.

The ex vivo model developed in this study has the advantage of being cost effective, reliable and easy to recreate. This means that similar models can be

used across a variety of applications to test burst strength allowing comparable testing of different techniques. This study used porcine oesophagus derived from animals used to provide tissue for medical training applications. During the development of the model fresh and fresh frozen oesophagus was used. Fresh frozen tissue was used in the final model due to availability but it is recognised that freezing tissue can cause damage that may alter the material properties however in the context of this study fresh frozen tissue was the only practicable solution. The advantage of sourcing the tissue from a single supplier who handles the tissue in the same way each time and then storing and handling the tissue in the laboratory in a consistent fashion is that variation within the model can be minimised. The disadvantage clearly stems from the fact that the freezing process may alter the material properties in a manner that influences the overall results.

6.3 Polymer

POSS PCLU was used in this study to develop the oesophageal repair patch. POSS PCLU is a degradable modification of POSS PCU a polymer developed at UCL and trademarked as UCL Nano. UCL Nano has been used in clinical applications to create replacement tear ducts and tracheal prostheses with varying success. The addition of the POSS nanoparticles allows the bulk material properties of the PCLU to be modified for the specific application. When selecting a polymer for this application a number of criteria needed to be met; the polymer and its dissolution products could not be directly toxic to the surrounding tissues and in the setting of oesophageal repair should not generate a local or systemic inflammatory response. The polymer should match the bulk material properties of the surrounding tissues including stiffness to reduce fibrosis. It is recognised that foreign material within the body will cause inflammation and fibrosis over a period of time and while the material properties of the polymer can be tailored to minimise this it was felt that a degradable polymer should be trialled. POSS PCLU is degradable compared to POSS PCU however this degradation in bulk properties is seen over a time period of several months, in the context of the duration of effect required in healing oesophageal injuries the degradation is not relevant.

6.4 Cell Behaviour

In this study cell culture experiments were used to evaluate the effect of a variety of polymer properties on the attachment and behaviour of cells. Cell culture experiments fundamentally rely on cell attachment and growth on tissue culture plastic, a polystyrene. The material properties and surface chemistry of polymers may enhance or diminish cell attachment and growth as might dissolution products.

The cell culture experiments performed in this study demonstrated reduced cell attachment and growth compared to tissue culture plastic overall. This differs markedly from established research on polyurethanes. Personal communication (Professor George Hamilton) suggested a possible explanation that the DMAC solvent may be responsible, this is highly cytotoxic and in some previous experiments POSS PCLU samples needed to be rinsed multiple times to remove any residual traces of the DMAC. This was not recognised during this series of experiments but may account for the results differing from much of the published literature. A solution to this problem in future work is to have a more robust protocol for washing samples beyond the sterilisation technique used and in addition samples could be left for longer in the oven curing to further remove DMAC by evaporation.

Specific properties of the polymer were compared including the thickness of the material which acted as a surrogate measure of stiffness and demonstrated little effect on cell behaviour. In the series of experiments examining the effect of altering surface chemistry cold plasma was used to successfully increase the wettability of the polymer. This has been shown to increase protein adsorption and increase cell adhesion and growth in other studies. In this study there appeared to be no effect on cell attachment or behaviour in the modified polymer samples however the potential presence of toxic DMAC may account for this finding.

6.5 Adhesives

An element of this study involved investigating techniques for attaching the patch to the oesophagus with the aim of sealing a perforation. The role of

adhesives in medicine is described in detail in previous chapters and commercially available fibrin glue was used for this study. This fibrin glue has the advantage of being readily available, reliable and approved for clinical applications. In contrast to the effect on cell adhesion and growth fibrin glue strength was increased following plasma surface modification to increase the wettability of the polymer surface. This is likely due to improved protein adsorption a well-described effect of increased wettability. The strength of the fibrin adhesive increased when incubated in cell culture media at 37^{o}C for one week suggesting that in vivo the fibrin glue would be effective over that period of time, this is likely to be important in a clinical application. As discussed above POSS PCLU will not degrade noticeably in the time period required for oesophageal leaks and perforations to heal. The duration of effect of the fibrin is much shorter and is likely to the dominant effect in a clinical setting. In a clinical setting it is likely that the polymer patch will detach from the oesophageal lining as the fibrin glue degrades and pass through the gastrointestinal tract.

This study has demonstrated the feasibility of the concept of a polymer patch attached to the oesophagus using fibrin glue and furthermore has offered a cost effective reliable model for testing oesophageal repair techniques. This concept is not yet suitable for clinical trials and it is important to identify what the next stages in this project might encompass.

6.6 Further work

This project has focused on development of a bench top model aiming to test some aspects of a novel endoscopically applied oesophageal patch. The next stage of this work needs to apply this technique in a reallife setting. Two approaches are possible, a live animal model of oesophageal perforation or a human trial that allows aspects of the patch to be tested prior to use in perforations.

As discussed in previous chapters animal models have inherent problems relating to ethics, cost and applicability although they have the advantage, in this context, of accurately recreating the injury and allowing explantation of

the patch along with the oesophagus to perform histological analysis. Human trials carry ethical and legal considerations but certain conditions mimic the scenario being tested, specifically endoscopic mucosal resection for early tumours and a patch could be tested in these patients, indeed similar technologies are already used in clinical practice[120]. Human trials and technology development fall under the remit of the Medicines and Healthcare Products Regulatory Agency (MHRA) and any human trials of an oesophageal patch would require approval from this body.

6.7 Conclusion

This study has looked at the development of a benchtop model testing aspects of a novel endoscopically placed polymer patch for the management of oesophageal leaks and perforations. The model is cost effective, reliable and gives reproducible results. Further work would look at either development of an animal model or moving to human trials in patients with appropriate pathology and clinical need.

7 References

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