Master of Philosophy (Surgery)

Thesis by Publication

Title:

# Aspects of Hernia Repair

Candidate:

Dr Paul David Patiniott, MBBS

Supervisory Panel:

Professor Guy Maddern (primary – 60%)

Dr Alex Karatassas (secondary – 40%)

Dr Prue Cowled (post-graduate co-ordinator)

Word Count: 17,000

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Paul David Patiniott - Signed Date: 29 November 2021
MBBS (Adel. 2012), MPhil (Surgery) Candidate
The University of Adelaide, The Queen Elizabeth Hospital, Department of Surgery

## Chapter 1: Introduction

## Chapter 2: Publication 1

Patiniott P, Reid J, Maloney RT, Karatassas A, Maddern G.

Elective abdominal wall hernia repair surgical mortality-A systematic review of the literature and peer review of mortality in Australia. *ANZ J Surg. 2021 Jun 14.*

## Chapter 3: Publication 2

Patiniott P, Stagg B, Karatassas A, Maddern G.

Developing a Hernia Mesh Tissue Integration Index Using a Porcine Model-A Pilot Study. *Front Surg. 2020 Nov 26.*

## Chapter 4: Publication 3

Jacombs ASW, Karatassas A, Klosterhalfen B, Richter K, Patiniott P, Hensman C.

Biofilms and effective porosity of hernia mesh: are they silent assassins? *Hernia. 2020 Feb;24(1):197-204.*

## Chapter 5: Publication 4

Patiniott P, Jacombs ASW, Kaul L, Richter K, Klosterhalfen B, Karatassas A, Maddern G

The role of bacterial biofilms in late hernia mesh complications. *Unpublished and Unsubmitted work written in manuscript style.*

## Chapter 6: Conclusions

# Chapter 1: Introduction

Abdominal hernia repair is one of the most common elective surgical procedures undertaken globally [1]. Although elective surgical repair of abdominal wall hernias is generally considered low risk, the procedures are so common that mortality trends begin to emerge and warrant evaluation. RACS ANZASM surgical mortality audits provide an independent, external peer review of surgical mortality in all states and territories of Australia. It is important to review all deaths that occur during an episode of surgical care and to provide opportunities for improvements in patient outcomes [2].

Although it is important to understand the factors contributing to elective hernia repair mortality, hernia mesh morbidity is also a concerning issue. A plethora of hernia mesh products are currently on the market, with more devices being introduced every year [3]. Despite this, there is a lack of evidence relating to how many of these commonly used mesh products perform on a biological level after they have been implanted into a patient. This in turn limits our ability to reliably predict the degree of tissue integration based on the specific type of mesh.

With so many prosthetics available, it can be difficult for surgeons to choose the most appropriate mesh for their patients [3]. As outlined by Klinge and Klosterhalfen there will never be one single ideal mesh for all purposes and mesh must be selected based on the specific functional requirements [4]. Successful hernia repair is not simply a technical exercise in covering a defect with a securely attached mesh; it mandates an understanding of how the patient’s inflammatory response influences surgical outcomes [5]. Failure to appreciate the importance of the biological aspect of hernia repair results in complications such as reduced effective porosity, biofilm formation, fibrosis, chronic mesh infection and pain [6]. Improving our understanding of the biological response elicited by any given mesh and the factors affecting this response will challenge existing assumptions about how mesh products help achieve successful surgical outcomes in patients.

In the pilot study, we utilised a large animal (porcine) model to develop a numerical Mesh Tissue Integration (MTI) Index focused on visible tissue ingrowth, fibrosis, adhesion formation and resorption of mesh as originally proposed by Karatassas et al. in 2018 [7]. Developing a functional, numerical MTI Index involved following

several of the guidelines set out by the International Organization for Standardization (ISO) which sets the standards for evaluation of biomaterials, in particular ISO 10993-6 which specifies test methods for the assessment of the local effects after implantation of biomaterials intended for use in medical devices [8].

The aim is to assist surgeons in selecting the most appropriate mesh according to its tissue ingrowth characteristics matched to the patient, adopting an evidence-based approach to achieve improved surgical outcomes and optimal patient-centred care. This thesis focuses on three key aspects of abdominal wall hernia repair.

Firstly, the epidemiological factors were investigated by means of conducting a systematic review of the literature regarding perioperative mortality in human adults undergoing elective surgical abdominal wall hernia repair and an audit of the Royal Australasian College of Surgeons (RACS) Australian and New Zealand Audit of Surgical Mortality (ANZASM) database to identify and evaluate the factors associated with perioperative mortality in Australian adults undergoing elective surgical abdominal wall hernia repair. With respect to the elective surgical repair of abdominal wall hernias in Australian adults, identifying the epidemiological factors associated with surgical related mortality informs more appropriate patient selection and improved patient care in our hospitals. [9]

Secondly, the current theoretical basis of abdominal wall hernia mesh repair was examined and tested scientifically by way of a pilot study utilising a porcine (pig) animal model to evaluate commonly used surgical hernia mesh devices, thereby constituting the basis for the development a functional Mesh Tissue Integration (MTI) Index.

Finally, by undertaking a systematic review of the existing literature on bacterial biofilms and hernia mesh, investigating the role of biofilms in hernia mesh complications by means of conducting investigations on explanted hernia mesh specimens that had been removed from human patients due to various clinical complications.

All three projects involved multi-disciplinary collaboration between surgeons, pathologists, scientists, engineers, biostatisticians, international experts and industry.

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Chapter 2: Statement of Authorship and Publication 1:

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Elective abdominal wall hernia repair surgical mortality - A systematic review of the literature and peer review of mortality in Australia |
| Publication Status | Published |
| Publication Details | ANZ J Surg. 2021 Jun 14. |

## Principal Author

| | |
|---|---|
| Name of Principal Author (Candidate) | Paul Patiniott |
| Contribution to the Paper | Conceptualisation, Literature Review, Data Collection and Analysis, Authorship, Review |
| Overall percentage (%) | 80% |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
| Signature | Date: 13 / 10 / 2021 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| | | | |
|---|---|---|---|
| Name of Co-Author | Jessica Reid | | |
| Contribution to the Paper | Co-authorship, Revision | | |
| Signature | | Date | 13/10/21 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Ryan Maloney | | |
| Contribution to the Paper | Co-authorship, Data Analysis | | |
| Signature | | Date | 19/10/2021 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Alex Karatassas | | |
| Contribution to the Paper | Supervision and Review | | |
| Signature | | Date | 12/10/21 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Guy Maddern | | |
| Contribution to the Paper | Supervision and Review | | |
| Signature | | Date | 13/10/21 |

# Introduction

Abdominal hernia repair is one of the most common elective surgical procedures undertaken globally.[1] In the United States more than one-million abdominal wall hernias are repaired annually with the majority of these consisting of inguinal hernias.[2] In Australia, statistics from the Australian Institute of Health and Welfare (AIHW) reported for elective admissions involving surgery, public and private hospitals 2015–2018 indicate that on average there are just over 60,000 elective hernia procedures undertaken annually in Australia with the slight majority of these being undertaken in private hospitals.[3] Although the mortality risk associated with elective hernia repair is low, even in the elderly, hernia is considered an avoidable cause of death.[4] There are currently no published systematic reviews on elective abdominal wall hernia related mortality in the literature.[5]

The purpose of this review is to identify the current evidence base on the factors which are most significantly associated with increased perioperative mortality in human adults undergoing elective surgical abdominal wall hernia repair. This in turn will provide context for the Royal Australasian College of Surgeons Australian and New Zealand Audit of Surgical Mortality (RACS ANZASM) an elective hernia repair mortality audit which aims to identify the epidemiological factors associated with surgical related mortality and inform more appropriate patient selection for elective hernia surgery.[6]

The second part of this paper examines information from the Australian and New Zealand Audit of Surgical Mortality (ANZASM), a peer reviewed database of surgical deaths under the governance of the Royal Australasian College of Surgeons (RACS). The objective of the audit is to improve the quality of surgical practice through information gathering, education and facilitation of change. The audit has been conducted Australia-wide since 2010, in this time substantial data on surgeon identified preventable issues contributing to in-hospital surgical deaths has been collected. While ANZASM was established for the purposes of both nation’s surgical communities, New Zealand does not currently participate in the ANZASM program.[7]

The first part of this paper includes a systematic review which seeks to answer the question of “which factors are most significantly associated with increased

perioperative mortality in human adults undergoing elective surgical abdominal wall hernia repair?”

The second component of this paper includes a retrospective review of ANZASM data which aims to identify how Australian mortality outcomes in elective abdominal wall hernia between 2012 to 2018 compare to the rest of the world. In particular the United States, given that currently the significant majority of published studies on this particular topic are predominantly comprised of retrospective analyses of data from the American College of Surgeons (ACS) National Surgical Quality Improvement Program (NSQIP)[8] and Veterans Affairs Surgical Quality Improvement Program (VASQIP)[9] databases.

## Methods

A systematic review was conducted in accordance with PRISMA guidelines for the reporting of systematic reviews and meta-analysis of observational studies.[10] Cochrane Library, PubMed, MEDLINE and Embase database searches and data extraction were conducted from June 1979 to October 2019.

The following search terms and MeSH headings were used and combined with AND operands: “elective,” “hernia,” “mortality.” Following de-duplication, initial titles and abstracts were reviewed to identify articles of potential interest; these were then retrieved in full-text format for review and data extraction.

**Table 1** Search terms utilised in the systematic review of the literature

| | Concept 1 | Concept 2 | Concept 3 | Concept 4 |
|---|---|---|---|---|
| Search terms | Surgical<br>Operative<br>Hernia<br>Repair Herniorrhaphy<br>Hernioplasty<br>Herniotomy | Abdominal wall<br>Ventral<br>Incisional<br>Epigastric<br>Umbilical<br>Ventral<br>Groin<br>Inguinal<br>Femoral<br>Spigelian<br>Lumbar<br>Obturator<br>Parastomal<br>Paraesophageal<br>Paraoesophageal<br>Hiatus<br>Hiatal<br>Diaphragmatic<br>Morgagni | Elective<br>NOT Emergency<br>NOT Acute<br>NOT Strangulation/ed<br>NOT Obstruction/ed | Mortality |

Cochrane Library, PubMed, Embase and MEDLINE databases were systematically reviewed

**Cochrane Library**

0 reviews or clinical trials for MeSH search Herniorrhaphy AND Mortality

No relevant studies when searching Hernia AND Mortality

**PubMed / MEDLINE**

Elective AND hernia AND mortality NOT emergency NOT acute – 254 results

Date: June 1979-October 2019, No Article Type Restriction

Subheading: mortality

MeSH Terms: hernia; mortality; elective surgical procedures

Filter Applied: Species: Humans – 234 results

Filter Applied: Adult 19+ Years – 156 results

Further Exclusion Criteria Applied –

Non-Adult (Ages < 18) (11)

Non-Hernia Surgery (47)

Non-Elective (15)

No Mortality Endpoint (11)

**Embase**

'elective surgery' AND hernia AND mortality NOT emergency

MeSH Search - “elective surgery” AND “hernia” AND “mortality” NOT emergency – 178 results

Filter: Study Type: Human – 175 results

Filter: Age: Adult – 96 results

Further Exclusion Criteria Applied

Non-Adult (0)

Non-Hernia Surgery (47)

Non-Elective (3)

No Mortality Endpoint (6)

Studies were included only if they were directly relevant to elective hernia surgery in an adult population and included mortality as an endpoint. Studies were required to include at least 100 subjects, and those explicitly describing early case experience or learning curves were not included to ensure that data were representative of an established practice. This approach was chosen to reduce the risk of selection bias from these studies. When two or more studies shared overlapping data sources, only one study was included according to the following prioritization criteria: the most detailed relevant outcomes, the largest patient population, and the most recent dataset. Study, patient, procedure, and outcome variables were extracted.

The function, governance and objectives of the ANZASM have been thoroughly described in other publications[11] however, a summary of the audit process can be described as follows. Independent of the treating surgeon, ANZASM is notified of in-hospital surgical deaths whether there was a surgical procedure or not by medical records departments. The hospital data includes designation of the admission as emergency or elective, which is determined by whether the admission was due to a planned procedure listed on a waiting list. Clinical details are collected via a standardised form completed by the treating surgeon either online or in paper format. ANZASM data is stored in an encrypted database that enables a complete audit trail. The de-identified patient details are sent for first-line assessment to a surgeon of the same specialty and from a different hospital. In most cases, conclusions may be reached about the patient's care at this point and further investigation is deemed unnecessary.

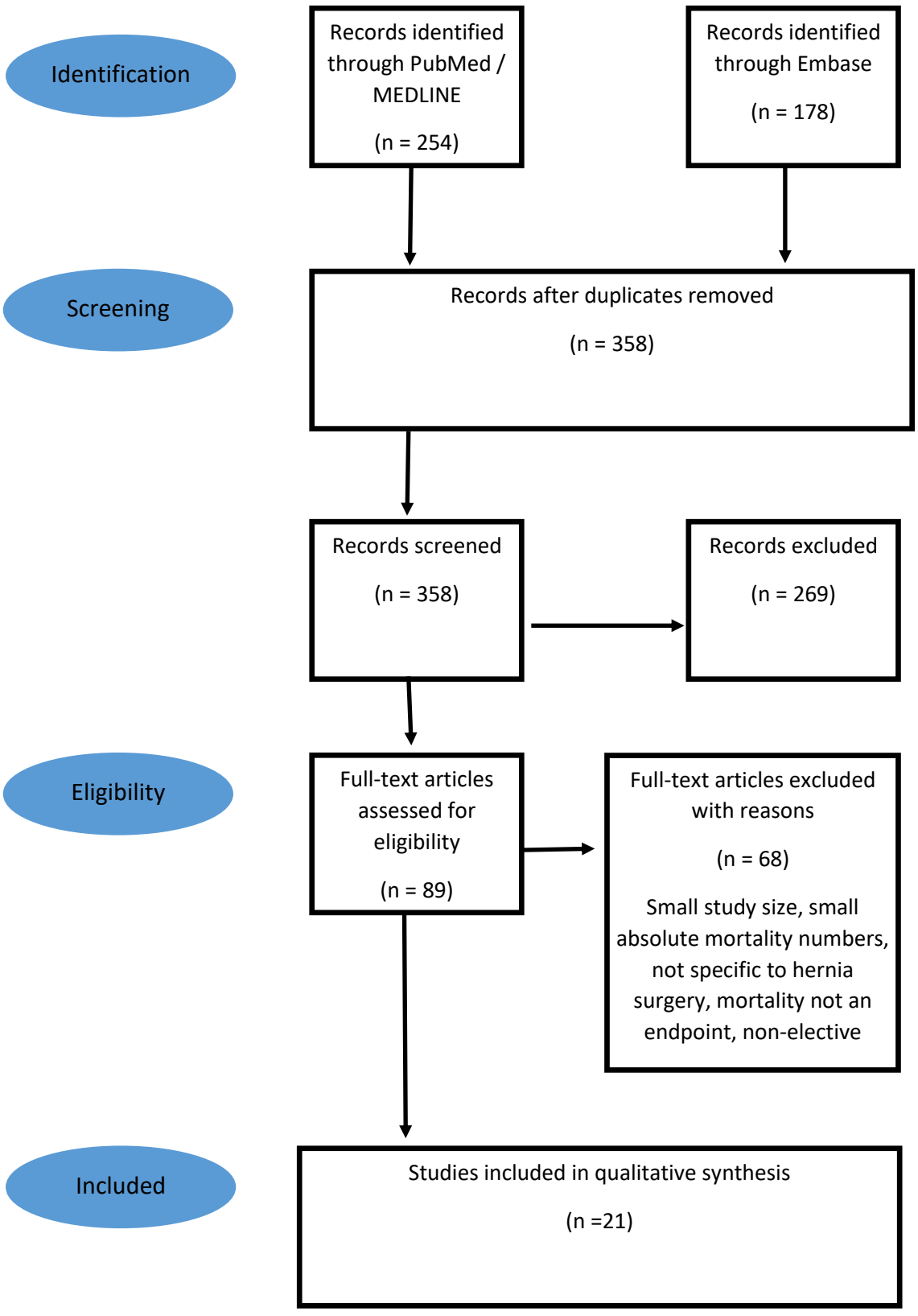
Alternatively, if further consideration of the patient's care is required, a second-line assessment is conducted by a different surgeon. The second-line assessor reviews the involved hospital's patient notes. Second-line assessment has been undertaken in 12–13% of all patients referred to ANZASM for the time period examined in this study.[12] As a part of the audit process, ANZASM assessors determine whether

there was an aspect of the patient’s management that could have been better, and feedback is provided to the treating surgeon.

The ANZASM data is held under qualified privilege which protects the confidentiality of identifying information for both practitioner and patient under the Australian Government Department of Health and Ageing (2014) Legislation for the Commonwealth qualified privilege scheme. This protection enables surgeons to freely divulge information with confidence that the information gathered will be used exclusively for the purposes of professional development. Since 2012, 100% of public hospitals, up to 92% of private hospitals and over 98% of surgeons participate.[12]

# Results

*Figure 1. PRISMA Algorithm*



Systematic review – *Table 2. Summary of the evidence*

| **Author** | **Year** | **Journal / Country** | **Publication Type** | **Population Size** | **Hernia Surgery Type / Population** | **Mortality Rate** |
|---|---|---|---|---|---|---|
| Khorgami Z, Hui BY, Sclabas GM | 2018 | Surg Endosc / USA | Retrospective review of a prospectively maintained database (HCUP-NIS) | 59,993 | Ventral<br>General pop. | Overall 0.2%<br>n = 135 |
| Ecker BL, Kuo LE, Simmons KD, et al. | 2016 | Surg Endosc / USA | Retrospective review of discharge data (California and New York) | 13,567 | Ventral<br>Lap vs Open<br>General pop. | Overall 0.24%<br>Lap 0.21%<br>Open 0.26%<br>No SS difference between groups (p = 0.56) |
| Bay-Nielsen M, Kehlet H. | 2008 | Acta Anaesth Scand / Denmark | Retrospective review of a prospectively maintained database. Danish Hernia Database (DHD) | 29,033 | Groin (Ing. + Fem.)<br>LA vs Regional (anaes) / General pop. | Overall 0.12%<br><65 years 0.015%<br>Higher mortality at 1 week observed in the regional anaesthesia group. Not SS. |
| Helgstrand F, Rosenber | 2013 | JACS / Denmark | Prospective nationwide study of the | 3,258 | Incisional<br>Lap vs Open | Overall 0.5% |

| | | | | | | |
|---|---|---|---|---|---|---|
| g J, Kehlet H, et al | | | Danish Ventral Hernia Database (DVHD) | | Young vs Old | 18-60y.o. 0.1% (2) n = 1,681<br>61-70y.o. 0.6% (5) n = 831<br>>70y.o. 1.2% (9) n = 746<br>SS difference between young vs old groups (p = 0.005)<br>No SS difference between lap and open (p = 0.52) |
| Ross SW, Oommen B, Huntington C, et al | 2015 | Am Surg / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 58,845 | Open Ventral / AWR<br>Plain Open vs Component Separation vs Panniculectomy vs Both CS + PAN<br>Complex AWR pop. | Overall 0.78%<br>n = 461<br>No SS difference between groups (p > 0.05) |
| Regner JL, Mrdutt MM, Munoz- | 2015 | Am J Surg / USA | Retrospective review of a prospectively maintained | 106,968 | Ventral<br>40% Normal vs 60% Obese pop. | Open 0.3% (106)<br>Lap 0.2% (27) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Maldonado Y. | | | database (ACS NSQIP) | | | No SS difference between groups (p = 0.4724) |
| Saleh F, Okrainec A, D'Souza N, et al. | 2014 | Am J Surg / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 37,645 | Primary Inguinal<br>Lap vs Open / General pop. | Open 0.05% (16)<br>Lap 0.02% (1)<br>No SS difference between groups (p = 0.34) |
| Cassie S, Okrainec A, Saleh F, et al. | 2014 | Surg Endosc / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 14,652 | Primary Umbilical<br>Lap vs Open / General pop. | Open 0.03% (4)<br>Lap 0.06% (1)<br>No SS difference between groups (p = 0.49) |
| Reynolds D, Davenport D, Roth JS. | 2013 | Surg Endosc / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 1,144 | Ventral<br>Functionally dependent pop. | Overall 3.8% (43)<br>Open 4% (40)<br>Lap 2.2% (3)<br>No SS difference between groups (p = 0.47) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mason RJ, Moazzez A, Sohn HJ, et al. | 2011 | Ann Surg / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 71,054 | Ventral / AWR<br>Lap vs Open / General pop. | 0.3% for both Open and Lap approaches<br>Open 0.33 (194)<br>Lap 0.18 (21)<br>No SS difference between open and lap groups in matched cohort after adjustment<br>(p = 0.754) |
| Sood RF, Lipira AB, Neligan PC, et al. | 2019 | Plast Reconstr Surg / USA | Retrospective cohort study | 2,283 | Ventral / AWR<br>General pop. | Overall 0.8% (18) |
| Nimptsch U, Mansk T. | 2015 | Dtsch Arztebl Int./ Germany | Retrospective review of nationwide hospital discharge data (DRG statistics) | 1,023,000 | All types<br>General pop. | Overall 0.13% (1316) |
| Spaniolas K, Laycock WS, | 2014 | Am J Surg / USA | Retrospective review of a prospectively maintained | 2,681 | Paraoesophageal<br>Laparoscopic | Old = 1%<br>Young = 0.4% |

| | | | | | | |
|---|---|---|---|---|---|---|
| Adrales GL, et al. | | | database (ACS NSQIP) | | Old vs Young Adult pop. | No SS difference between groups (p = 0.16) |
| Smolevitz J, Jacobson R, Thaqi M, et al. | 2018 | Am J Surg / USA | Single-centre retrospective review (Rush University Medical Center) | 185 | Complex Ventral / AWR<br>Class III Obese pop. | BMI >40 = 1.6%<br>BMI <39.9 = 3.4%<br>No SS difference between groups |
| Tam SF, Au JT, Chung PJ, et al. | 2015 | Hernia / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 700 | Ventral<br>Chronic dialysis patient pop. | Overall 0.2%<br>n = 188<br>Dialysis 1.4% (10)<br>Non-Dialysis 0.2% (178) |
| Ong W, Shen T, Tan WB, et al. | 2016 | Surg Endosc / Singapore | Prospective cohort study | 1,841 | Inguinal<br>On aspirin patient pop. | 0%<br>n = 0 |
| Benarroch-Gampel J, Sheffield KM, Duncan CB, et al. | 2012 | Ann Surg / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 73,596 | All Types<br>Pre-op blood test vs no blood test pop. | Overall = 0.3%<br>No SS difference between groups |
| Novitsky YW, | 2013 | Hernia / USA | Retrospective review of 2004-2008 Nationwide | 78,348 | Ventral<br>Hospital type / High vs Low | Only odds ratios provided |

| | | | | | | |
|---|---|---|---|---|---|---|
| Orenstein SB. | | | Inpatient Sample (NIS) database. | | Socioeconomic pop. | Overall 2.12 (1.43–3.13)<br>Medicare 2.16 (1.50–3.13)<br>Medicaid 2.04 (1.15–3.61) |
| Ross SW, Oommen B, Kim M, et al. | 2014 | Surg Endosc / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 6,841 | Ventral<br>Laparoscopic<br>Resident vs Junior vs Senior Surgeon | 0.3% for all groups<br>No SS difference between groups<br>p>0.05 |
| Schlosser KA, Kao AM, Zhang Y, et al | 2019 | Hernia / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 48,955 | Ventral<br>MELD-Na score<br>Non-cirrhotic pop. | 0.5%<br>n=253 (215 open, 38 lap) |
| Newman KL, Johnson K, Cornia P, et al. | 2019 | Gastroenterology / USA | Retrospective review of a prospectively maintained database (ACS NSQIP) | 7,869 | All types<br>Cirrhotic pop. | 0.6-1.2% |

The ANZASM study included elective hernia repair deaths between 2012 and 2018 of which there were 106 reported cases in total. Data was obtained from all states and territories in Australia, with the exception of New South Wales. The majority of cases were reported in Victoria (38) followed by Western Australia (26), Queensland (19), South Australia (15), Tasmania (6), Australian Capital Territory (1) and Northern Territory (1), the numbers are consistent with the population size for the respective states and territories.

There was a male (65 cases (61.3%)) to female (41 cases (38.7%)) preponderance, and an age range between 36 and 94 years, with an average mortality age of 76.7. The average length of hospital stay was 16.7 days. The highest mortality association was with ASA grade 3 (57), followed by grade 2 (21), grade 4 (18) and grade 1 (2), ASA status was unspecified in 8 cases.

The leading cause of death was cardiogenic including ischaemic heart disease, acute myocardial infarction, congestive cardiac failure, acute pulmonary oedema and cardiac arrest (24), this was followed by respiratory causes including aspiration, bronchopneumonia and respiratory failure (19). Sepsis and multi-organ failure (16) and direct surgical complications including anastomotic leaks, perforation, peritonitis (12) were the next most common cause of mortality in the examined cases. There was one (1) death due to pulmonary embolism and cause of death was not specified in 29 cases.

54 cases were recorded as open (50.95%) in contrast to 20 laparoscopic (18.87%), 2 cases were laparoscopic converted to open with the remaining 30 cases unspecified. With respect to mortality by type of hernia repair procedure, incisional hernia repair was the type most commonly associated with mortality (41) (primary 35 / recurrent 6), followed by inguinal (34) (primary 30 / recurrent 4), paraoesophageal (13), primary umbilical (10), primary ventral (7) and femoral (1). There was insufficient information included in the database to adequately further differentiate the incisional group into hernia subtype.

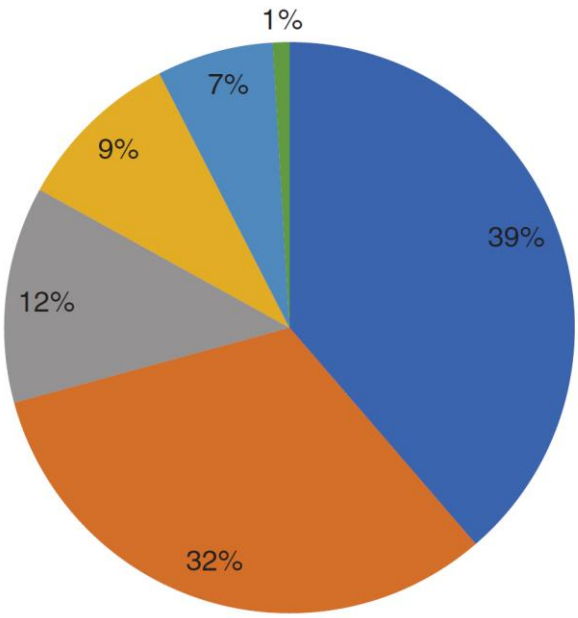



**Fig 2.** Elective hernia surgery mortality as per hernia type (■, incisional; ■, inguinal; ■, paraoesophageal; ■, umbilical; ■, ventral; ■, femoral).

**Table 3** Binary logistic regression to provide the risk adjustment (by age and sex) including all relevant predictors and two-way interactions

| | | Deceased | | |
|---|---|---|---|---|
| Predictors | Odds ratios | CI | Statistic | *p* |
| Intercept | 0.00 | 0.00–0.00 | −19.17 | **<0.001** |
| Year | 0.95 | 0.92–0.99 | −2.78 | **0.005** |
| Sex (male) | 0.67 | 0.11–4.28 | −0.43 | 0.666 |
| Age | 1.11 | 1.09–1.13 | 11.55 | **<0.001** |
| Sex (male)* Age | 1.00 | 0.97–1.02 | −0.27 | 0.785 |
| Observations | 597 362 | | | |
| $R^2$ Tjur | 0.002 | | | |

CI, confidence interval. Bold values indicate strong statistical significance

Statistical analysis utilising denominator values for elective hernia procedures derived from Australian Institute of Health and Welfare (AIHW) data. Data from each of 12 dataset blocks (05-06 to 16-17 financial year blocks) were loaded, filtered and concatenated. NSW data is omitted since NSW is also lacking from the previously provided ANZASM data.

Risk-adjusted peri-operative mortality rates for the relevant procedures are also produced, using a binary logistic regression for the risk-adjustment. Risk adjustment is based on patient age and sex, not admission status since the data only included elective procedures. Even before risk-adjustment, the probability of death in these procedures is already very low.

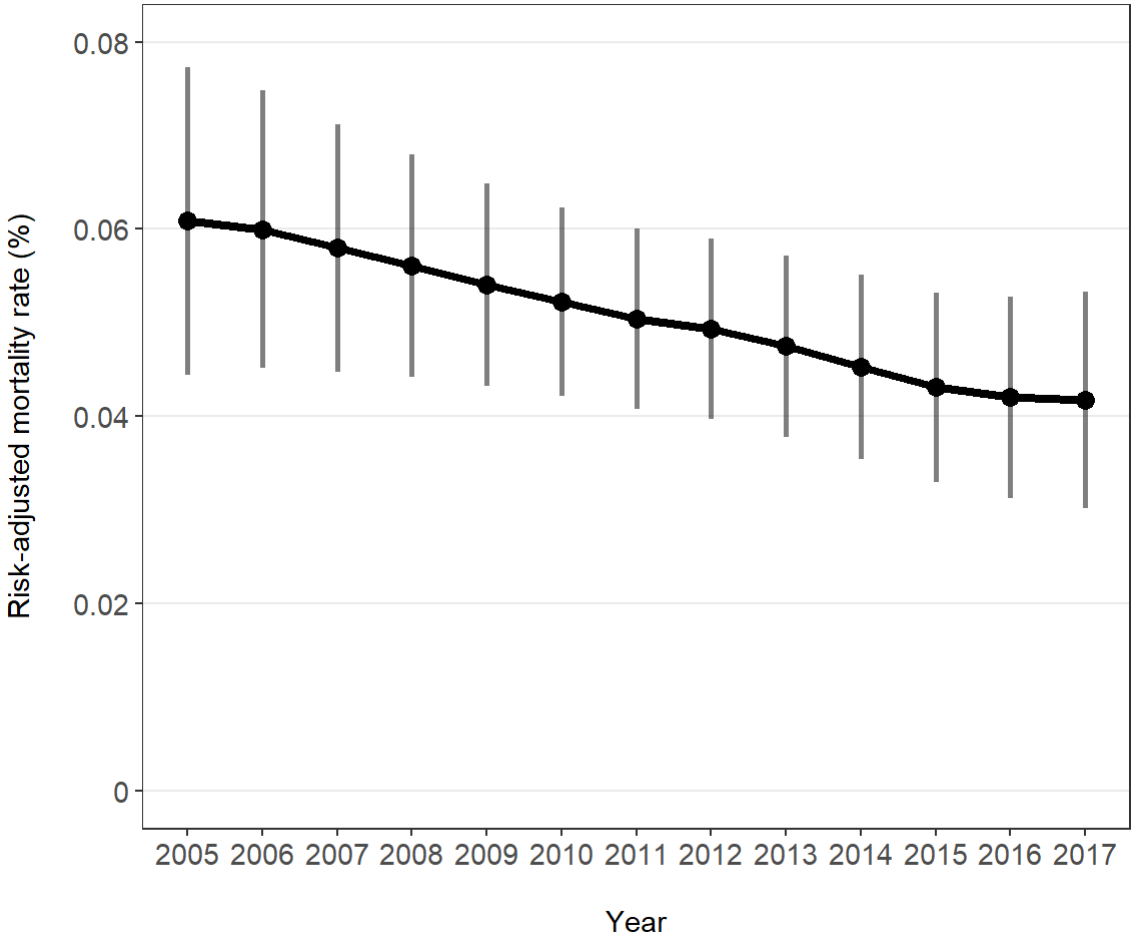



*Figure 3. Risk-adjusted mortality rate for elective hernia procedures according to separation year. Error bars show 95% confidence intervals for the rates, derived from the standard error of the model predictions.*

Through systematic review of the literature, it was established that the overall reported perioperative mortality in human adults undergoing elective surgical abdominal wall hernia repair was low (0.1-0.5%). By comparison, in our study looking at ANZASM and AIHW data, the calculated risk-adjusted mortality rate was found to be even lower between (0.04-0.06%).

## Discussion

The weight of evidence from the systematic review supported that there was no significant mortality benefit between open and laparoscopic surgical techniques, this was found to be the case independent of hernia site, type and co-morbidities. [13-22]

Most of the studies found that older age was associated with a higher risk of death[13,19,23,24] whereas Spaniolas and colleagues did not find a significant mortality difference between older and younger age groups in their 2014 study involving laparoscopic paraoesophageal hernia repair[25].

Congestive heart failure, chronic pulmonary and hepatic disease, neurological disorders and paralysis were associated with a higher mortality risk.[13,19,24] Obesity was associated with an increased risk of respiratory failure[23] but not mortality [26]. Dialysis patients had a significantly greater rate of 30-day perioperative mortality[27]

A Danish study by Bay-Nielsen and others found that regional anaesthesia was disproportionately more often used in patients dying within 1 week post-operatively.[22] The continuation of aspirin during elective hernia surgery did not have a significant effect on mortality.[28] Routine laboratory testing for elective hernia repair not recommended as neither testing nor abnormal results were associated with increased perioperative mortality.[29]

In addition to patient comorbidities, socioeconomic factors and hospital characteristics appeared to be major determinants of post-herniorrhaphy

complications and mortalities.[30] The seniority of operating surgeon however did not appear to result in a significant effect on the mortality rate.[31]

Several of the included studies utilised NSQIP data from overlapping time periods however there were considerable differences in study aims, target population and hernia subtype, thereby reducing the likelihood that the same patients were reported on more than once.

The main strength of the systematic review was that most of the studies included large population numbers with sound statistical analysis. A major limitation however was that most of the included studies were retrospective analyses of the NCS NSQIP / VASQIP databases coming from the United States of America, with very few significant studies coming from other countries.

With respect to the ANZASM study, strengths included utilisation of a prospectively maintained national surgical mortality database with multiple datapoints and comprehensive descriptions, the calculated mortality rates were statistically significant. The main limitation of this study was the inaccessibility of mortality of data from the state of New South Wales, this equates to being unable to present mortality data from approximately 30 percent of the total Australian population.

## Conclusion

We found that the risk-adjusted mortality rate for elective abdominal wall hernia surgery in Australia is very low and compares favourably to international statistics. Despite the low absolute numbers, the factors which were most significantly associated with increased perioperative mortality in patients undergoing elective surgical abdominal wall hernia repair included higher age, cardiorespiratory co-morbidity and incisional hernia repair. The findings of this study support adopting a judicious approach when advising this subgroup of patients on the risks and benefits of proceeding with elective abdominal wall hernia repair surgery.

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Chapter 3: Statement of Authorship and Publication 2:

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Developing a Hernia Mesh Tissue Integration Index Using a Porcine Model-A Pilot Study. |
| Publication Status | Published |
| Publication Details | Front Surg. 2020 Nov 26. |

## Principal Author

| | | |
|---|---|---|
| Name of Principal Author (Candidate) | Paul Patiniott | |
| Contribution to the Paper | Conceptualisation, Literature Review, Principal Investigator, Data Collection, Data Analysis, Authorship, Review | |
| Overall percentage (%) | 80% | |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. | |
| Signature | Date | 13 / 10 / 2021 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| | |
|---|---|
| Name of Co-Author | Brendan Stagg |
| Contribution to the Paper | Data collection and analysis, Co-authorship |
| Signature | Date: 13/10/2021 |

| | |
|---|---|
| Name of Co-Author | Alex Karatassas |
| Contribution to the Paper | Conceptualisation, Co-Investigator, Co-Author, Supervision and Review |
| Signature | Date: 12/10/21 |

| | |
|---|---|
| Name of Co-Author | Guy Maddern G. |
| Contribution to the Paper | Supervision and Review |
| Signature | Date: 13/10/21 |

# Introduction

Repairs of abdominal wall hernias are the most frequently performed operations in general surgery (1). The last 50 years has seen rapid advances in our understanding of the biological basis of hernia development, surgical technique for repair and, most significantly, the use of prosthetics (2). Although exact figures are unknown, it is estimated that more than 20 million prosthetic meshes are implanted worldwide each year (3, 4).

Several clinical studies have demonstrated the advantages of mesh implantation and therefore international guidelines recommend, independent from the surgical technique, the use of meshes in groin and ventral hernia repair (5–7). Today almost all groin hernias are treated with meshes (8) and the use of a prosthetic material for the surgical repair of abdominal wall hernia has almost universally become accepted as the current standard of practice (9, 10).

The modern-day surgeon is confronted with a plethora of different prosthetics from numerous manufacturers, and each year sees further meshes introduced to the market. With so many prosthetics available, it can be difficult for surgeons to choose the most appropriate mesh for their patients (2). Currently, there is no universal model that is used to compare mesh products. Blatnik and others advocate for standardized mesh labelling (11). Useful as this may be, we suggest that this information (i.e., size, composition, pore size, weight, biomechanical properties) in isolation is inadequate to form a basis for selecting which mesh is most optimal for use in our patients. The presumption is that this information can be extrapolated to predict tissue response to mesh and patient outcomes. However, several recent important studies support the notion that there is a fundamental gap in understanding the degree to which a mechanical mismatch between hernia repair materials and host tissue contributes to failure at the biomaterial-tissue interface (12).

A 2012 review into hernia mesh materials by Bilsel et al. recommends that in most instances surgeons should opt for a lightweight monofilament mesh, with large pores and minimal surface area (13). However, as outlined by Klinge and Klosterhalfen in 2013 there will never be one single ideal mesh for all purposes and mesh must be selected based on the specific functional requirements. Klinge and Klosterhalfen,

describe the concept of “effective porosity.” Meshes are designed with a certain porosity which may significantly decrease due to axial load, mechanical mismatch with host tissue and instability of the polymer composition. Their research has shown that pore sizes of over 1,000 microns result in less host inflammatory reaction and less fibrosis with improved mesh integration and less scar plate formation resulting in a superior repair (14).

Factors influencing the early efficacy of a hernia repair include adequate closure of the defect, the size and strength, weight of mesh, and the type and security of the mesh fixation. Longer-term efficacy is dependent on tissue incorporation into the scaffold of the mesh, the degree of mesh tissue ingrowth affects the hernia recurrence rate, the resistance of mesh to chronic infection and tissue flexibility relevant to the functional outcome.

To assist surgeons in mesh selection we aim to develop a numerical mesh-tissue integration (MTI) index as originally proposed by Karatassas et al. (15). Analysis was performed using specific macroscopic, microscopic, and biological testing techniques based on several of the established guidelines from the International Organization for Standardization (ISO). The ISO sets the standards for evaluation of biomaterials, in particular part 10993-6 which specifies test methods for the assessment of the local effects after implantation of biomaterials intended for use in medical devices (16).

The aim of this proof-of-concept pilot study was to investigate the viability of this method utilizing a porcine animal model to develop a viable MTI Index. This index will function as a standardized tool to assist surgeons in selecting the most appropriate mesh according to tissue ingrowth characteristics matched to the patient—a scientific, reproducible evidence based approach to achieving improved surgical outcomes in hernia patients.

## Methods

This was a large animal (porcine) pilot study to evaluate the safety and efficacy of commonly used mesh products for the treatment of abdominal wall hernia in patients in turn providing the scientific foundation and appropriate model for the development

of a functional MTI index.

The relevant institutional ethics approval was obtained, and the study was conducted at The Large Animal Research and Imaging Facility (LARIF) of the South Australian Health and Medical Research Institute (SAHMRI) located at Gilles Plains, South Australia.

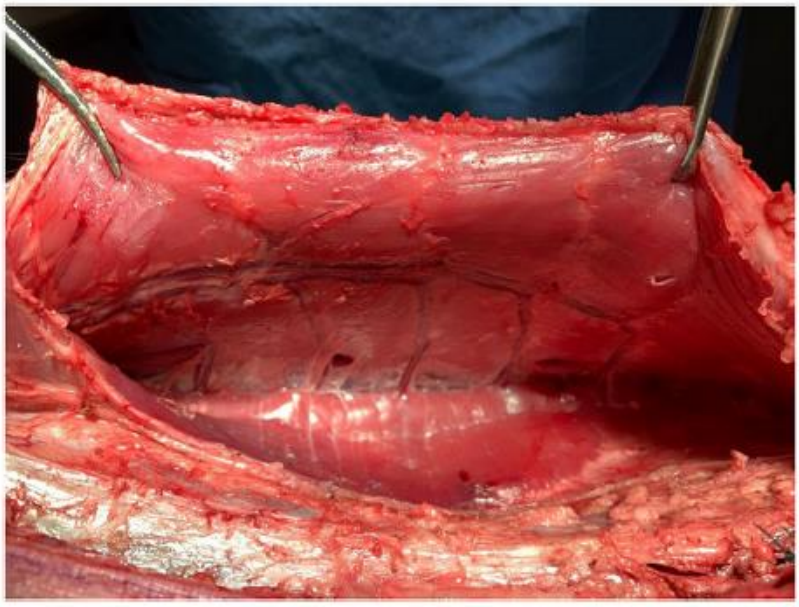

**FIGURE 1** | Rectorectus plane with inferior epigastric vessels and perforator bundles near linea semilunaris.

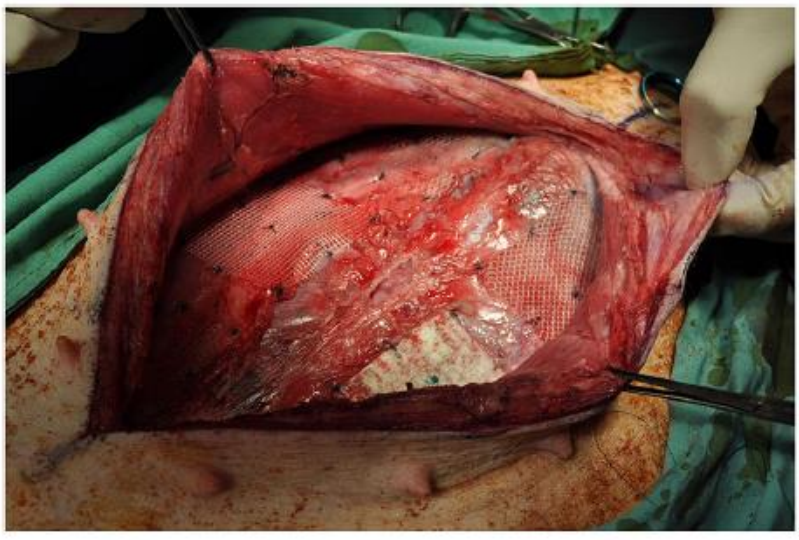

**FIGURE 2** | Subrectus implantation of mesh devices.

Two white female Landrace pigs weighing 37 and 40 kg, respectively, were enrolled in this study. The animals were allowed to socialize and acclimatize to the facility for 2 weeks prior to undergoing any procedures. They had access to fresh water, nutrition on a calorie-restricted diet to avoid excessive weight gain, and daily care provided by the specialist SAHMRI animal team under the supervision of a veterinarian. The pigs were assessed for wellness 24 hours prior to surgery, then fasted from 12 hours prior to surgery. Sedation was achieved with ketamine (15mg/Kg, IM) injection and the animals subsequently anesthetized using oxygen-isoflurane inhalation. Each animal was intubated for approximately 3 h (Figure 1).

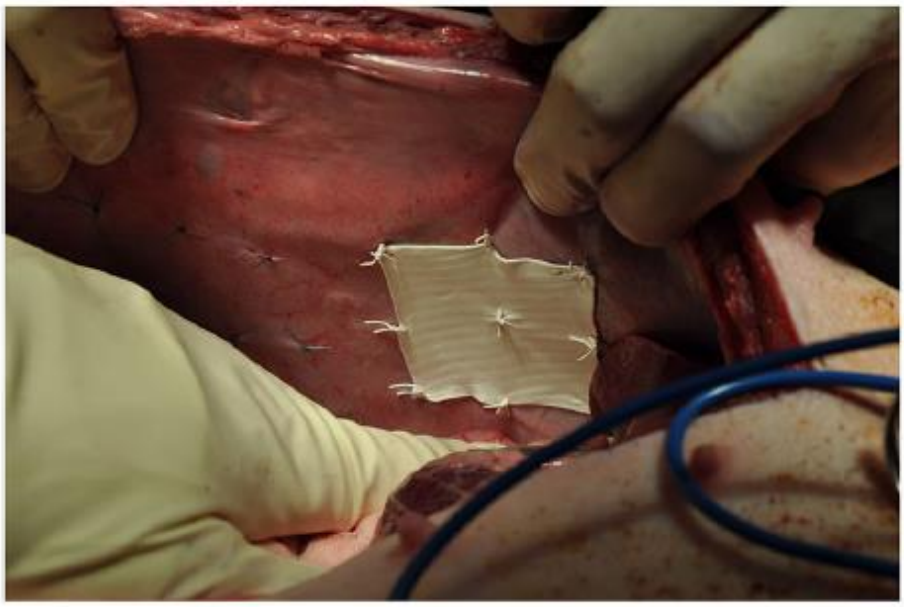

**FIGURE 3** | Positioning of intraperitoneal mesh (ePTFE) and control.

The abdomen was shaved, washed with povidone iodine and draped for surgical sterility. A 30 cm midline incision was made to gain access to the abdomen. On both sides of the abdomen a sub rectus plane was developed. Two pieces of mesh (5 × 5 cm) biosynthetic, polyester and a control were implanted in the sub rectus space on the left side of the abdomen and three pieces (5 × 5 cm) polyethylene terephthalate, polyester and polypropylene on the right side. The mesh squares were separated by 5 cm. A further six meshes (5 × 5 cm) were inserted intraperitoneally, lateral to the rectus muscle; three on the left side of the abdomen, three on the right side. All meshes were secured with 9 sutures preventing folding of mesh which may influence porosity (Figure 2).

A total of 8 different mesh devices and 2 controls were surgically implanted in subrectus and intraperitoneal tissue planes. The controls consisted of 5cm x 5cm designated areas where 9 sutures were applied without mesh. The procedure was replicated identically for both pigs. (Figures 3, 4)

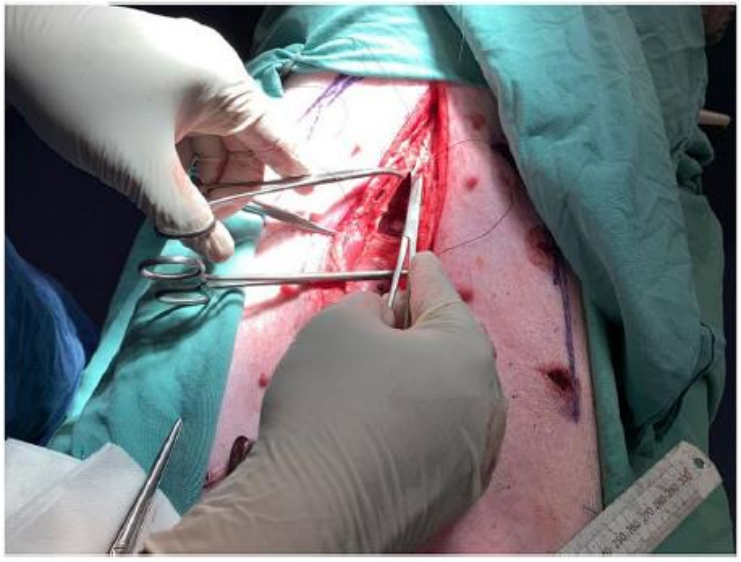

**FIGURE 4 |** Closure of posterior layer after implantation of intraperitoneal mesh.

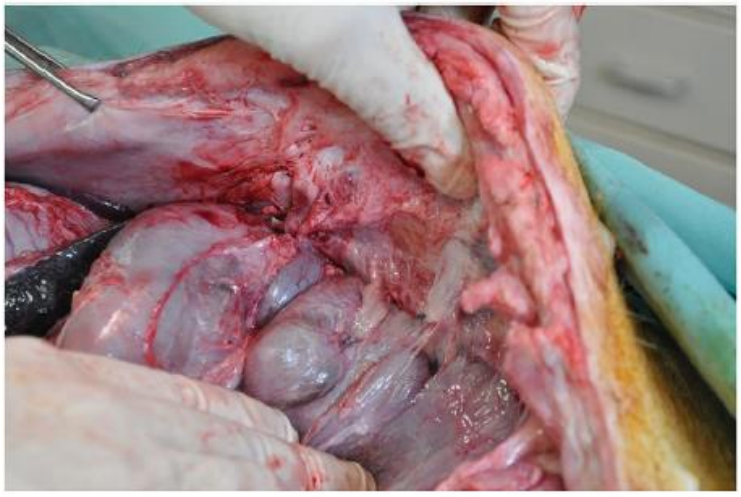

**FIGURE 5 |** Post-mortem intraperitoneal macroscopic assessment for adhesions between mesh and viscera.

The pigs were euthanised utilising 15mLs of Lethabarb (Pentobarbitone Sodium 325mg/mL) via intravenous cannula. The first was euthanised at 2 weeks post-implantation weighing 40kg and second pig at 4 weeks post-implantation weighing 48.5kg. This was to facilitate the explantation of the abdominal wall and subsequent preparation of the mesh-tissue specimens for macroscopic, histological and biomechanical analysis. (Figures 5, 6)

The surgeons then performed a laparotomy and completed the assessment and recording of adhesion scores, the abdominal wall was separated from the animal in its entirety. Careful dissection was undertaken to define the mesh-tissue specimens which were subsequently excised from the abdominal wall and securing sutures removed. Pathology and engineering teams were blinded to the mesh being evaluated. The specimens were not labelled by brand name but instead given codes corresponding to the anatomical plane and location. For example, the mesh placed in the most anterior position on the left side of the subject in the pre-peritoneal plane was labelled PL-1, the mesh placed in the most anterior position on the right side of the subject in the intraperitoneal plane was labelled IR-1. This labelling was consistent between both pigs with the only difference being that the location of the intraperitoneal control was varied from IR-1 to IR-2 for Pig 1 and Pig 2 respectively.

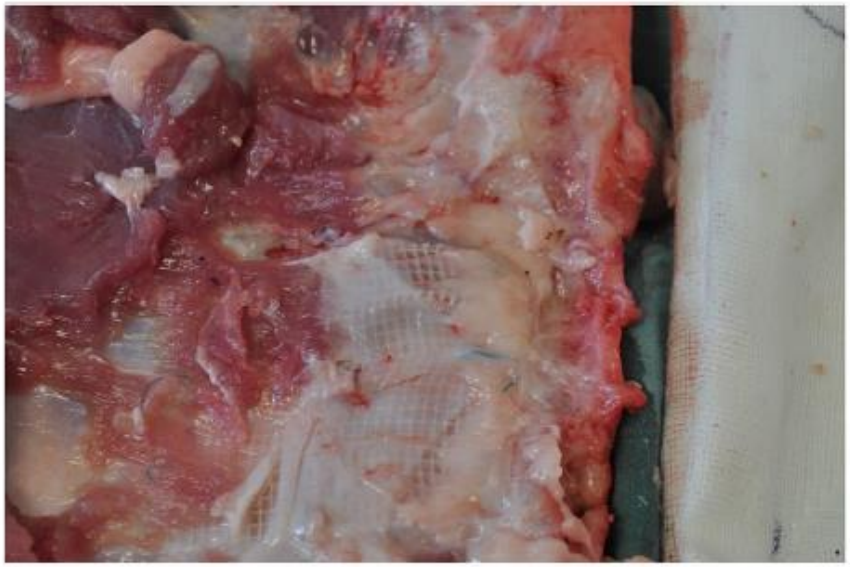

**FIGURE 6 |** Macroscopic tissue integration of polyester mesh, subrectus plane at 2 weeks post implantation.

The handling of biological specimens including mesh and tissue is potentially hazardous. Moreover, investigators must be wary of the inherent risk of disrupting the tissue architecture due to poor handling techniques. In order to mitigate these risks, the wearing of full sterile personal protective equipment (PPE) was enforced whenever handling specimens. In addition to this, the respective mesh-tissue specimens were carefully placed in specialized pathology containers to protect their structural integrity throughout the transportation and storage process.

After the relevant macroscopic, histological, and biomechanical testing had concluded, the specimens were disposed of in the relevant medical biohazard waste disposal units in accordance with institutional guidelines which are subject to state legislation as per the Environmental Protection Act (EPA) of 1993. The following scoring system was utilized for standardizing macroscopic assessment.

1. Visualization of degree of tissue incorporation into mesh
    1. 75–100% of mesh visible (minimal coverage of mesh with tissue)
    2. 50–75% of mesh visible
    3. 25-50% of mesh visible
    4. 0–25% mesh visible (mesh nearly or completely covered by tissue)
2. Degree of mesh shrinkage
    1. 50% shrinkage
    2. 30–50% shrinkage
    3. 10–30% shrinkage
    4. <10% shrinkage’
3. Degree of adherence - force required to distract mesh from tissue
    1. Pulls away from tissue with minimal force (easily detaches with forceps)
    2. Pulls away from tissue with moderate force (detaches with use of artery forceps)
    3. Pulls away from tissue with firm force (amount of force required may partially tear mesh)
    4. Firmly attached. Cannot be pulled away

4. Adhesions to mesh—Adhesion scoring method derived from Lauder et al. (pig) Standardized grading for adhesions to be assessed by blinded independent surgeon (17). Adhesion characteristics

0. No adhesions
1. Thin filmy adhesions
2. More than one thin adhesion
3. Thick adhesions with focal point
4. Thick adhesions with planar attachment
5. Very thick vascularised adhesions or more than one planar adhesion.

For the purpose of microscopic analysis A 50 × 10mm strip of tissue was harvested from the center of each mesh or control site, and an orienting suture was placed at the cranial end of the specimen. After fixation in 10% neutral buffered formalin for a minimum of 12 h, the entire craniocaudal aspect of the specimen was sectioned for histological assessment. A standard 14 h processing cycle was performed, and the specimen was embedded such that the relationship between the mesh and underlying tissue layers could be examined in the plane of section. The paraffin blocks were sectioned at 4 microns, and a routine hematoxylin and eosin stain was performed.

The slides from each specimen were reviewed at scanning magnification, and formal histological assessment was performed in areas where the mesh was well-oriented and the tissue reaction was representative of the specimen as a whole. The caliber and amount of space between mesh fibers varied significantly between specimens. As such, histological assessment was performed at the mesh-tissue interface at the deep aspect of the specimen.

Scoring was performed as per the guidelines of the International Organization for Standardization (ISO). ISO sets the standards for evaluation of biomaterials, specifically, ISO 10993-6 Biological evaluation of medical devices—Part 6: Tests for local effects after implantation. For each specimen, cell indices were derived as an average of 10 consecutive high power fields using a 40x objective with a field diameter of 0.52 mm (Table 1).

20 × 50mm fresh sections were excised from the respective explanted mesh-tissue specimens and taken to The University of Adelaide, North Terrace campus for the purpose of biomechanical testing. The University Department of Engineering designed and constructed a custom-made pin loaded clamp for use with the Instron Model 1011 testing machinery, securing the tissue in order to determine the precise force (Newtons) vs. displacement (mm) curve associated with the distraction of mesh from tissue for each of the extraperitoneal and intraperitoneal mesh products in Pig 1 and Pig 2.

**TABLE 1** | Histological evaluation scoring system–cell type/response.

| Cell type/Response | 0-None | 1–Minimal | 2–Mild | 3–Moderate | 4–Severe |
|---|---|---|---|---|---|
| Polymorphonuclear cells | 0 | Rare, 1–5/phf | 5–10/phf | Heavy infiltrate | Packed |
| Lymphocytes | 0 | Rare, 1–5/phf | 5–10/phf | Heavy infiltrate | Packed |
| Plasma cells | 0 | Rare, 1–5/phf | 5–10/phf | Heavy infiltrate | Packed |
| Macrophages | 0 | Rare, 1–5/phf | 5–10/phf | Heavy infiltrate | Packed |
| Giant cells | 0 | Rare, 1–2/phf | 3–5/phf | Heavy infiltrate | Sheets |
| Necrosis | 0 | Minimal | Mild | Moderate | Severe |
| | | | Score | | |
| **Response** | **0** | **1** | **2** | **3** | **4** |
| Neovascularisation | 0 | Minimal capillary proliferation, focal 1–3 buds | Group of 4–7 capillaries with supporting fibroblastic structures | Broad band of capillaries with supporting structures | Extensive band of capillaries with supporting fibroblastic structures |
| Fibrosis | 0 | Narrow band | Moderately thick band | Thick band | Extensive band |
| Fatty infiltrate | 0 | Minimal amount of fat associated with fibrosis | Several layers of fat and fibrosis | Elongated and broad accumulating of fat cells about the implant site | Extensive fat completely surrounding the implant |

## Results

Macroscopically, there was no difference in visual MTI scores at 2 weeks, there was minor shrinkage of the polyethylene mesh and adherence scores were generally higher in the subrectus plane with the exception of the biosynthetic mesh. The manufacturer of the biosynthetic mesh recommends pre-peritoneal placement of this product, intraperitoneal placement was not recommended (Table 2).

At 4 weeks, macroscopic MTI scores had improved for all the devices with favorable adherence, minimal to zero shrinkage, and fibrosis with the exception of PR-3 polyester, which scored comparatively lower in the 3 key domains (Table 3).

**TABLE 2 |** Macroscopic assessment–Pig 1 (2 weeks).

| Mesh | MTI | Fibrosis/ Shrinkage | Adherence | Adhesions (Intraperitoneal) |
|---|---|---|---|---|
| PR-1 (control) | Control | Control | Control | – |
| PR-2 biosynthetic | 1 | 4 | 3 | – |
| PR-3 polyester | 1 | 4 | 3 | – |
| PL-1 polyester & polylactic acid (PLA) | 1 | 3 | 4 | – |
| PL-2 polyester | 1 | 4 | 3 | – |
| PL-3 polypropylene | 1 | 4 | 3 | – |
| IR-1 ePTFE | 1 | 4 | 1 | 0 |
| IR-2 (control) | Control | Control | Control | 0 |
| IL-1 biosynthetic | 1 | 4 | 4 | 2 |
| IL-2 polyester | 1 | 4 | 1 | 0 |

**TABLE 3 |** Macroscopic assessment–Pig 2 (4 weeks).

| Mesh | MTI | Fibrosis/ Shrinkage | Adherence | Adhesions (Intraperitoneal) |
|---|---|---|---|---|
| PR-1 (control) | Control | Control | Control | – |
| PR-2 biosynthetic | 2 | 4 | 3 | – |
| PR-3 polyester | 1 | 4 | 2 | – |
| PL-1 polyester & polylactic acid (PLA) | 3 | 3 | 4 | – |
| PL-2 polyester | 2 | 4 | 3 | – |
| PL-3 polypropylene | 3 | 4 | 3 | – |
| IR-1 (control) | Control | Control | Control | 1 |
| IR-2 ePTFE | 1 | 4 | 1 | 1 |
| IL-1 biosynthetic | 3 | 4 | 4 | 3 |
| IL-2 polyester | 1 | 4 | 1 | 1 |

There was no substantial difference in adhesive strength between mesh-tissue specimens observed at 2 weeks (Figure 7). However, at 4 weeks clear trends began to emerge. Significantly, mesh devices implanted in the pre-peritoneal plane required much higher average and peak loads (Newtons) to distract the mesh from tissue when compared to the products implanted in the intraperitoneal plane (Figure 8, Table 4).

Histological changes were apparent in all devices at 2 weeks and even more pronounced at 4 weeks. The biosynthetic and polypropylene devices displayed the highest histological scores at 4 weeks followed by polyester. Lower histological scores were associated wth the intraperitoneal ePTFE and composite (barrier) mesh devices (Table 5).

**TABLE 4 |** Histology results–pig 1 (2 weeks).

| Pig 1 (2 weeks) | PR-1 *control* | PR-2 biosynthetic | PR-3 polyester | PL-1 polyester + PLA | PL-2 polyester | PL-3 polypropylene | IR-1 ePTFE | IR-2 *control* | IL-1 biosynthetic | IL-2 polyester |
|---|---|---|---|---|---|---|---|---|---|---|
| **Inflammatory** | | | | | | | | | | |
| Neutrophils | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| Lymphocytes | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 1 | 2 | 3 |
| Plasma cell | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| Macrophages | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 1 | 3 | 3 |
| Giant cells | 0 | 3 | 3 | 1 | 2 | 1 | 3 | 0 | 3 | 1 |
| Necrosis | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| Subtotal | 6 | 12 | 13 | 10 | 10 | 9 | 9 | 2 | 8 | 9 |
| **Other changes** | | | | | | | | | | |
| Fibrosis | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| Fat infiltration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Neovascularisation | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 1 | 2 |
| Subtotal | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 1 | 2 | 4 |
| Total | 8 | 14 | 15 | 12 | 12 | 12 | 11 | 3 | 10 | 13 |

**TABLE 5 |** Histology results–Pig 2 (4 weeks).

| | PR-1 *control* | PR-2 biosynthetic | PR-3 polyester | PL-1 polyester + PLA | PL-2 polyester | PL-3 polypropylene | IR-1 *control* | IR-2 ePTFE | IL-1 biosynthetic | IL-2 polyester |
|---|---|---|---|---|---|---|---|---|---|---|
| **Inflammatory** | | | | | | | | | | |
| Neutrophils | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 1 |
| Lymphocytes | 0 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 |
| Plasma cell | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Macrophages | 0 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 |
| Giant cells | 0 | 3 | 3 | 1 | 1 | 2 | 0 | 1 | 3 | 1 |
| Necrosis | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Subtotal | 0 | 11 | 9 | 7 | 7 | 11 | 2 | 6 | 10 | 8 |
| **Other changes** | | | | | | | | | | |
| Fibrosis | 0 | 1 | 1 | 3 | 1 | 1 | 4 | 1 | 1 | 2 |
| Fat infiltration | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Neovascularisation | 0 | 1 | 3 | 2 | 3 | 2 | 1 | 1 | 2 | 2 |
| Subtotal | 0 | 3 | 4 | 6 | 5 | 3 | 5 | 2 | 3 | 4 |
| Total | 0 | 14 | 13 | 13 | 12 | 14 | 7 | 8 | 13 | 12 |

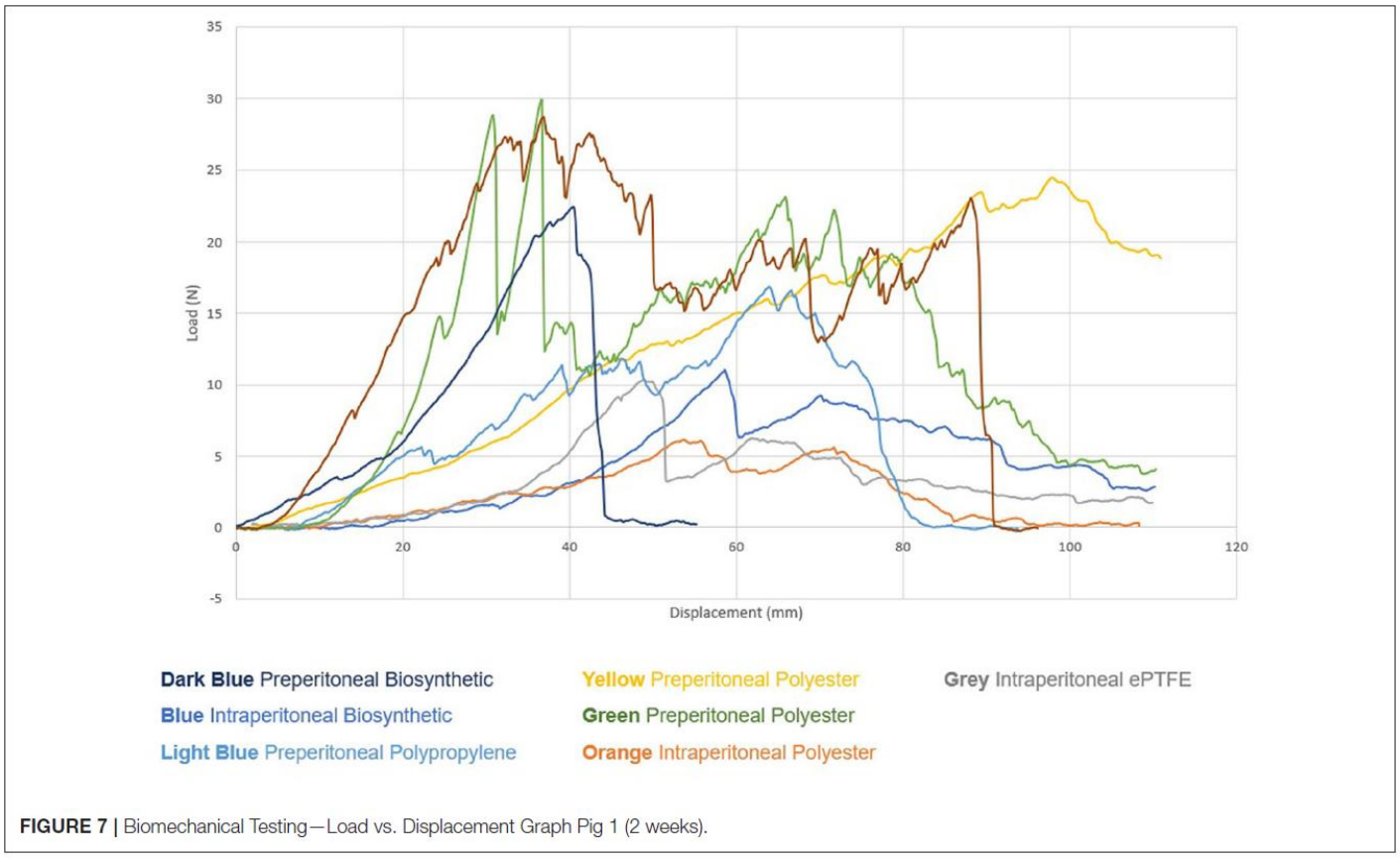


**FIGURE 7 |** Biomechanical Testing—Load vs. Displacement Graph Pig 1 (2 weeks).

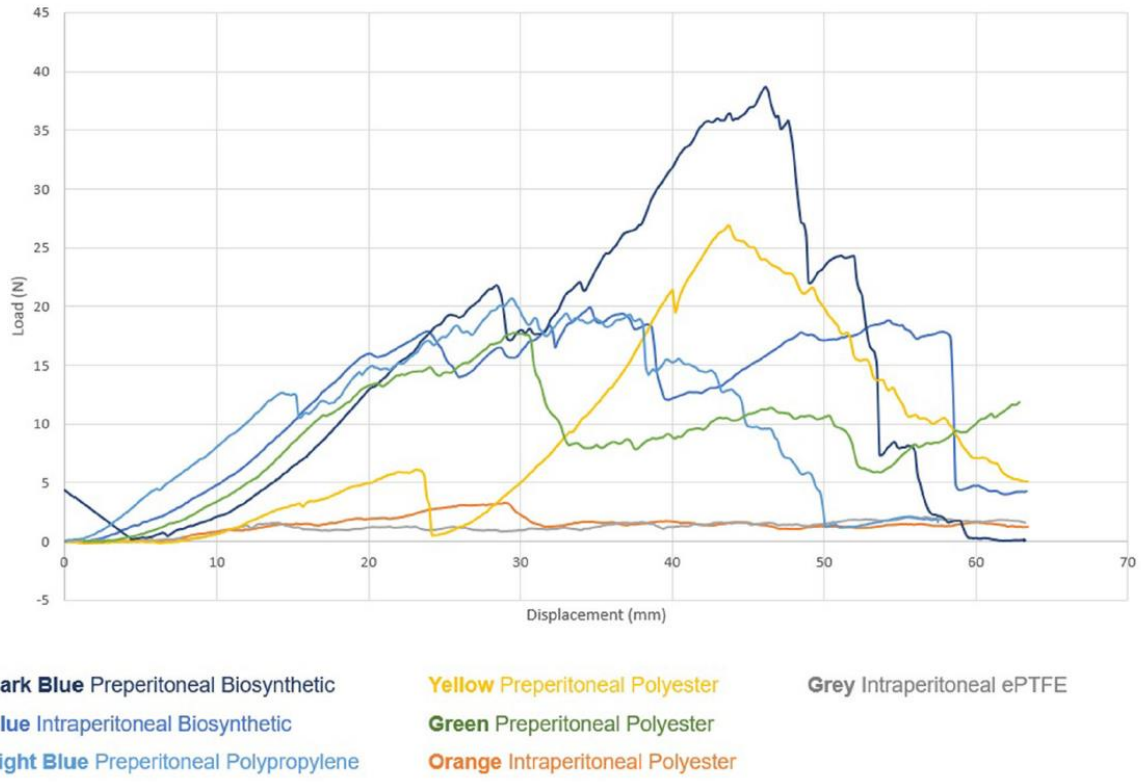


**FIGURE 8 |** Biomechanical Testing—Load vs. Graph Pig 2 (4 weeks).

## Discussion

Significant differences between the degrees of MTI were observed at 2 weeks and the distinctions were even more apparent at 4 weeks. The experimental protocol was kept as identical as possible for both timeframes, this was an intentional feature of the study design. The rationale for doing this was to minimize the number of confounding variables when investigating the changes to MTI scores over time whilst still allowing for direct comparison of the various biomaterials. One of the interesting incidental findings observed in this study is that mesh products placed in the subrectus plane displayed greater degrees of adhesion strength and integration than those placed intraperitoneally.

Microscopic assessment was limited by substantial variation in mesh microarchitecture between products. For example, IR-1 appeared to contain a continuous band of mesh with no perceptible gaps, whereas PL-2 contained 1mm spaces between mesh fibers. The amount of space between mesh fibers appeared to limit the amount of room for tissue infiltration (Figure 9).

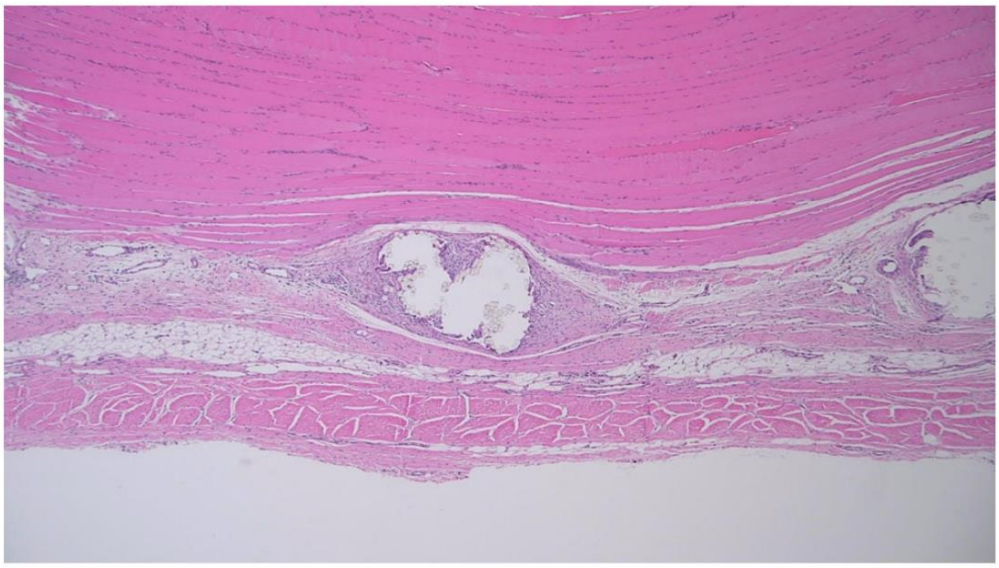

**FIGURE 9 |** Specimen PR-2 viewed at 40× magnification (Pig 2).

As such, the tissue response was assessed at the deep surface of the mesh to promote consistent comparison between specimens. However, for parameters such as neovascularisation and adipose tissue formation, it was more practical to examine the tissue between mesh fibers. Efforts to standardize microscopic assessment between specimens were limited by variation in mesh microarchitecture (Figure 10).

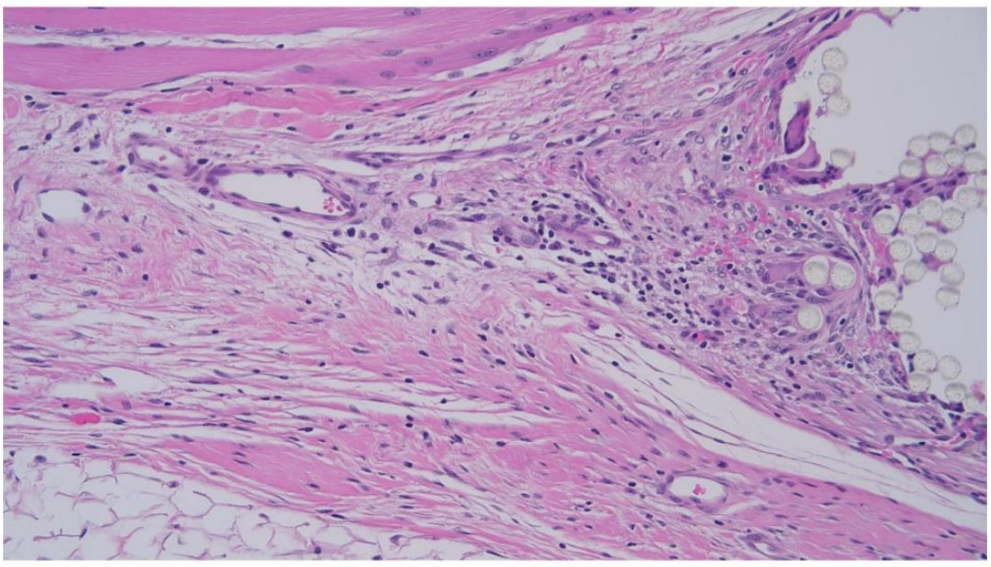

**FIGURE 10 |** Specimen PR-2 viewed at 200× magnification (Pig 2).

Macroscopic analysis of the subrectus mesh demonstrated that the MTI scores were on average higher in Pig 2 (4 weeks) in comparison with Pig 1 (2 weeks), whereas the lower MTI scores for the intraperitoneal mesh remained largely unchanged between the 2 pigs, with respect to both fibrosis and adherence. If additional pigs were to be recruited in future studies and endpoints were extended to 8 and 12 weeks or even longer, more datapoints would be generated and we may expect to see more significant differences in both fibrosis and adherence scores for these intraperitoneal meshes.

The lack of improvement in MTI scores for the intraperitoneal mesh products between Pig 1 and 2 is not unexpected as according to the theory of effective porosity, the barrier protection significantly reduces effective porosity and hence rate and degree of MTI. No significant adhesions were noted in the intraperitoneal meshes in both pigs but this may change with longer term studies. In future studies the Jenkins scale (18), a standardized and validated adhesion scoring system could also be considered as a viable alternative to the Lauder scoring system which was utilized in this study.

Biomechanical testing and analysis of the explanted mesh tissue specimens showed good correlation with the macroscopic index scores, however in this study there was no clear correlation with the histological findings.

Key strengths of this study included that the porcine model has proven to be very sound for the purposes of challenging our hypothesis and developing a MTI index. The study design facilitated multiple mesh products and a control to be tested simultaneously without any discernible detrimental effects to the animals. The ability to correlate results between macroscopic, microscopic and biomechanical analyses was desirable, with good concordance between macroscopic and biomechanical domains. As collaboration between investigators from several disciplines was required, blinding was essential to reducing the risk of investigator bias.

Some of the weaknesses of this study included small numbers, with only two pigs having been used in this pilot study, larger numbers in future studies will reduce the risk of bias and increase the validity. Although 4 weeks was adequate for the purposes of demonstrating model viability, ideally observation over a longer

timeframe with several later study endpoints i.e. to 3, 6, or 12 months will likely result in the observation of greater variability between index scores across several domains and the emergence of stronger trends and relationships between variables. In retrospect, it was concluded that the study was made significantly more technically difficult as a result of mesh products being implanted in two anatomical planes in the same animal. Ensuring that overlapping of mesh devices did not occur was unnecessarily challenging. In future studies, appropriate consideration should be given to modifying the study to only involve implantation of mesh in one anatomical plane per animal. Future studies may also benefit from including an investigation into the effect of adjuncts on MTI; in 2007 Fortelny et al. described how a cyanoacrylate tissue sealant reduced the effective mesh porosity thereby having a detrimental effect on (MTI) (19). Conversely, in 2008 Petter-Puchner et al. utilized a rodent model to examine a fibrin-based tissue sealant which displayed a favorable MTI and adhesion profile, although more data and a longer observational period would have strengthened the study (20).

Recently, there has been increasing scrutiny in the media of hernia mesh products on an international level, public pressure on governments has resulted in the relevant regulatory bodies upgrading the classification of hernia mesh products mandating a higher degree of regulation in line with other medical prosthetic devices such as orthopedic joints and cardiac implantable devices. In view of recent events highlighting the risks associated with the use of surgical mesh devices The Australian Government Therapeutic Goods Administration (TGA) has recognized this deficiency in monitoring and regulation and recently strengthened their assessment of surgical mesh medical devices by approving regulatory amendments that reclassified all these medical devices from Class IIb (medium) to Class III (high risk) (21).

There are several successful established registries of surgical outcomes in other disciplines, including the Australian Orthopedic Association National Joint Replacement Registry (AOANJRR) (22) and the Australian Breast Device Registry (ABDR) (23) amongst others. It is lamentable that such a registry has not yet been established for hernia mesh prostheses in the field of hernia surgery.

The importance of establishing a longitudinal patient database and hernia mesh registry cannot be overstated, it will enable the identification of specific patient

conditions and surgical factors which influence the tissue integration process and clinical outcome. It will also allow for the evaluation of adjuncts, such as tissue ingrowth promoters and therapies, such as those targeting the formation of biofilms. Further it will serve as a guide to surgeons when selecting the most appropriate mesh product for their hernia patient, facilitating optimal patient centered care.

The development of a hernia MTI index as proposed in this pilot study used in conjunction with hernia mesh registries are likely to become increasingly important references for mesh manufacturers when providing preregistration data to the relevant regulatory authorities prior to market release and subsequent clinical utilization.

Longer term studies will facilitate the development of a degradation index to supplement the integration, fibrosis and adhesion indexes. It is envisaged that the MTI Index will be a useful tool for individualizing hernia treatment for patients, the ultimate intention for this model is that it will be utilized synergistically with long term mesh-patient outcome registries and databases to inform improved matching of mesh to patient, particularly in the setting of the complex hernia repair and abdominal wall reconstruction.

It is important to emphasize that this is a pilot study providing the framework for a proof-of-concept MTI index study which will involve increasing the number of subjects and observing integration over a longer timeframe. We propose proceeding to involving 10 pigs over 3 months, a larger number of data points will be essential if this is to be considered a significant model.

## Conclusion

This pilot study is one of the first to propose a functional, biological standardized model for comparing hernia mesh products. The results are encouraging and demonstrate that this is potentially a robust and transferrable model for assessing MTI in hernia mesh. A proof-of-concept study involving larger numbers and longer study endpoints is required to further improve the validity of this model.

## Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics Statement

The animal study was reviewed and approved by South Australian Health & Medical Research Institute (SAHMRI) Project No. SAM323.

## Author Contributions

PP: contributed to the majority of study design, implementation, and manuscript writing.

BS: responsible for the histological analysis and production of histology results and tables, reviewed paper, and contributed to the discussion section.

AK: research supervisor to PP, responsible for ethics approval, study design, study implementation, and manuscript review/editing.

GM: primary research supervisor to PP, responsible for research governance, oversight, and manuscript review/editing.

All authors contributed to the article and approved the submitted version.

## Funding

The research was funded by a grant from W. L. Gore & Associates, Inc. which was administered by The University of Adelaide.

## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chapter 4: Statement of Authorship and Publication 3:

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Biofilms and effective porosity of hernia mesh: are they silent assassins? |
| Publication Status | Published |
| Publication Details | Hernia. 2020 Feb;24(1):197-204. |

## Principal Author

| | | | |
|---|---|---|---|
| Name of Principal Author | Anita Jacombs | | |
| Contribution to the Paper | Principal Author | | |
| Overall percentage (%) | 70%. | | |
| Certification: | This paper reports on original research I conducted during the period of Paul Patiniott's Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. | | |
| Signature | | Date | 13/10/2021 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| | | | |
|---|---|---|---|
| Name of Co-Author | Alex Karatassas | | |
| Contribution to the Paper | Co-authorship, Review, Supervision | | |
| Signature | | Date | 12/10/21 |

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|---|---|---|---|
| Name of Co-Author | Katharina Richter | | |
| Contribution to the Paper | Co-authorship, Review | | |
| Signature | | Date | 13/10/2021 |

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|---|---|---|---|
| Name of Co-Author | Paul Patiniott | | |
| Contribution to the Paper | Co-authorship, Systematic Literature Review | | |
| Signature | | Date | 13/10/2021 |

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|---|---|---|---|
| Name of Co-Author | Bernd Klosterhalfen | | |
| Contribution to the Paper | Co-authorship, Review | | |
| Signature | | Date | 10/26/2021 |

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|---|---|---|---|
| Name of Co-Author | Chris Hensman | | |
| Contribution to the Paper | Co-authorship, Review | | |
| Signature | | Date | 13th October 21 |

# Introduction

The impact of bacterial biofilms and effective mesh porosity on hernia repair is unstudied topics to date. A literature review was conducted and although there are numerous studies relating to biofilms in orthopaedic and breast prosthesis, there is a paucity of literature pertaining to surgical mesh biofilms. Further, the review supports that effective porosity is a relatively recent concept.

Having major implications on disease conditions, bacterial biofilm infection and mesh porosity may present a plausible explanation for delayed “mesh infection” and failure of hernia repair as a result of fibrosis and contracture of the mesh. It is imperative that surgeons are familiar with these two conditions and implement appropriate measures to study and manage them.

## Literature Review Objectives

We undertook a review of the current literature on bacterial biofilm in hernia mesh and effective porosity.

## Literature Review Methods

Ovid Medline and PubMed were searched for communications on “effective porosity” and “bacterial biofilm”. Study eligibility criteria included peer-reviewed journals written in the English language, articles published between 1990 and 2019 were considered.

Search terms included “biofilm” AND “mesh”, a secondary search was conducted with the terms “effective porosity” AND “mesh”. Studies were selected based on their relevance to the study question and excluded if they did not meet eligibility or relevance criteria. Included articles were then individually reviewed and selected. Risk of bias was not evaluated for the purposes of this literature review.

# Results

A search of the term biofilm in isolation yielded 46,751 results. When refined to biofilm AND mesh this significantly reduced the number to 129 results, 124 of which were written in English language. These 124 articles were individually reviewed and

of these only 13 articles were deemed to be peer reviewed and directly relevant to the topic of surgical mesh and biofilms (Fig. 1).

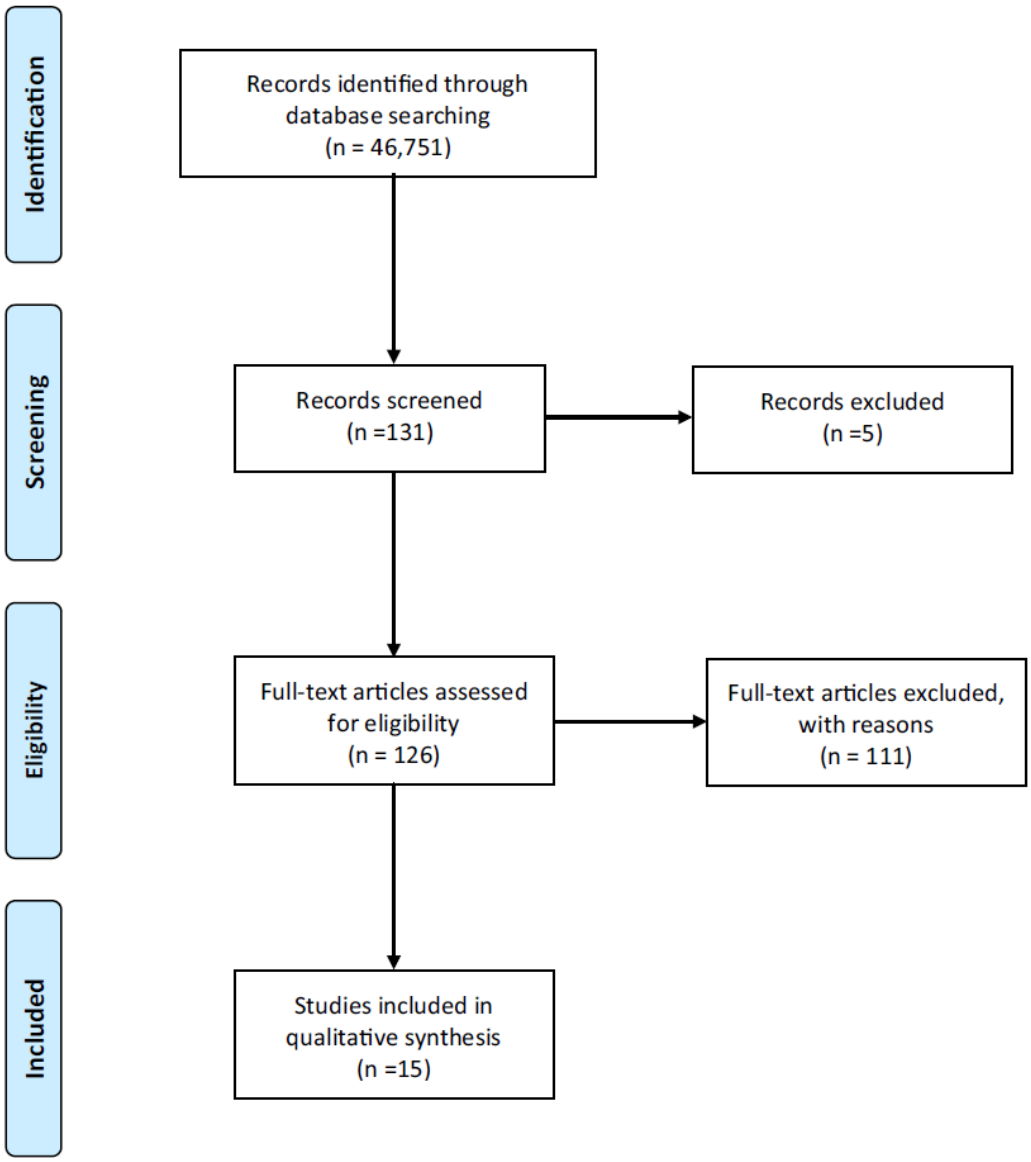


**Fig. 1** Prisma flow chart of the selection process for the included studies

## Summary

The literature on biofilms and surgical mesh is relatively recent. In 2008 Engelsman et al. [1] were amongst the first to publish their findings on effect of surgical mesh material and morphology on biofilm growth. Similar observational studies by Aydinuraz [2] and Relinsky [3] involving incubation, colony quantification ensued. In 2012 Stoodley and colleagues [4] utilised confocal microscopy and fluorescent in situ hybridization (FISH) to further characterise the interaction of bacterial biofilms with

surgical mesh in vitro [5] and then subsequently in vivo [6]. Several publications which followed from these initial studies focused on prevention and treatment strategies in relation to biofilms in surgical mesh. Jacombs [7] and Reinbold [8] evaluated antibiotic impregnated mesh products, Cazalini [9] investigated the capacity of metals such as Tungsten-DLC to reduce biofilm formation and Bigelow [10] histotripsy. Landmark review articles by Perez-Kohler [11] in 2016 and Guillaume and colleagues in 2018 [12, 13] provided valuable insights and brought to light the scope of challenges when it comes to evaluation and treatment of biofilm and mesh.

## Definition and pathogenesis

Biofilms are defined as “aggregates of microorganisms in which cells, that are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPS), adhere to each other and/or to a surface” (IUPAC definition) [14]. Bacterial biofilms are formed when bacteria convert from their planktonic (“free-floating”) genetic phenotype to a sessile genetic phenotype [15]. This occurs frequently in both nature and human disease, as the biofilm formation provides the bacteria with a significant survival advantage. Once the biofilm producing phenotype has been activated the bacteria begin to produce a protein–polysaccharide-rich matrix of EPS which encloses them [16, 17]. This 3-dimensional biofilm matrix provides mechanical stability for the embedded bacteria and physical protection from external stressors (Fig. 2). The EPS matrix can prevent or limit the activity of immune cells and therapeutic compounds entering or diffusing through the matrix, hence providing a protected haven for bacteria to survive and reproduce [16].

Conversely the EPS matrix facilitates bacterial communication, important for reproduction and survival, and promotes exchange of genetic information, including antibiotic resistance genes [16, 17]. Hence new antibiotic-resistance genes often rapidly spread through bacteria within biofilm [17]. Together the physical barrier and swift integration of novel antibiotic-resistance genes make the eradication of entire biofilm challenging even with best medical interventions [15, 16, 18].

Research has shown that biofilms require antimicrobial concentrations up to 1000-fold higher to kill biofilm bacteria than compared with their planktonic counterparts, often at levels toxic to the human host [16]. Also, most antimicrobial compounds are less effective against biofilms due to rise and spread of antibiotic resistance in the biofilm [17];the agents failing to penetrate the biofilm matrix in significant concentrations to be biologically active [16]; the agents are inactivated and degraded by bacterial enzymes released into the biofilm matrix [19] and most antibiotics target active bacteria, hence, the agents are not effective against bacteria with reduced metabolic activity and slow growth (common in the sessile state) [16].

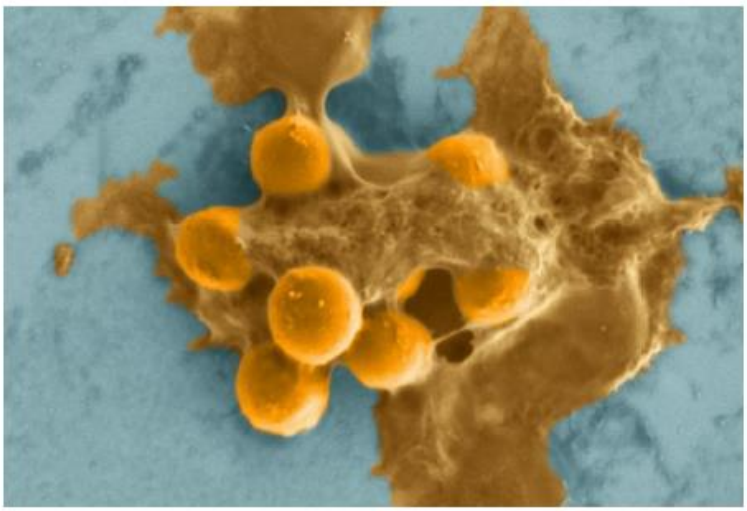

**Fig. 2** *Staphylococcus aureus* biofilm. Scanning electron micrograph of bacterial clusters embedded in a protective matrix. Image provided by Dr. Katharina Richter [27]

Biofilm-producing bacteria have predilection for adhering to surfaces, which is frequently observed in clinical practice [15, 16]. Bacterial biofilms have been found on most implantable, abiotic surfaces used in medicine and surgery, including temporary implants (for example intravenous catheters, indwelling urinary catheters, ureteric stents) and permanent implants (for example orthopaedic implants, breast implants, cardiac pacemakers) [15, 20–22]. Bacterial biofilm infection of luminal implants, including venous catheters, urinary catheters, ureteric stents, arterial stents, frequently results in luminal obstruction and is the main source of infective emboli [23–25]. Thus, biofilm infection is the main aetiology for removal of these implants when they become obstructed or in the setting of sepsis. There is now increasing evidence that bacterial biofilm infection of surgical implants results in chronic inflammation and correlates with implant failure of several surgical implants.

A causal relationship between bacterial biofilm formation and implant failure has been well demonstrated with orthopaedic implants (knee and hip prostheses) resulting in chronic osteomyelitis and delayed loosening of implant and breast implants resulting in capsular and breast deformity [19, 22].

Bacterial species implicated in biofilm disease are very similar for both orthopaedic and breast implant diseases. Skin commensals such as coagulase negative Staphylococci species, particularly Staphylococcus epidermidis and S. aureus, are frequently linked to biofilm-related orthopaedic and breast implant infections [19, 22]. Implant contamination has been shown to occur mainly at the time of insertion, when the risk of implant contact with patient's own skin is highest [19, 22]. Implant contamination after insertion, such as during episodes of bacteraemia, has been demonstrated but is very uncommon.

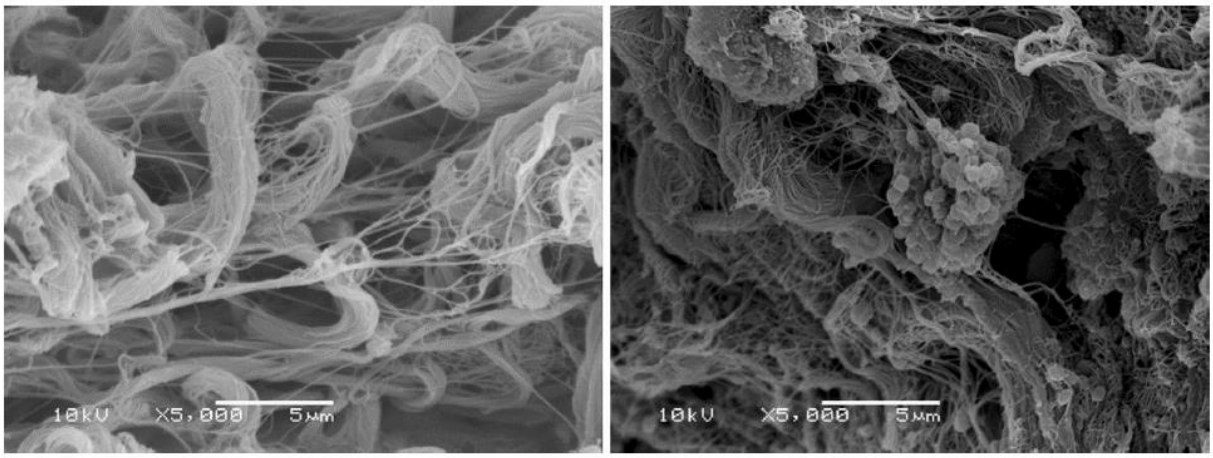


**Fig. 3** Scanning electron micrographs of in vivo human periprosthetic biofilm. Human breast capsules after explanation of breast implant and complete capsulectomy. Left: normal fibrous capsule with not showing normal fibrous structure with no bacterial cells or biofilm formation. Right: biofilm-infected human breast capsule with numerous coccoid bacteria attached to the capsule fibres and to each other with EPS in dense colony (central structure) and scattered throughout capsule fibres. Images provided by Dr. Anita Jacombs

Biofilm infections of joint prostheses and breast implants develop slowly with patients manifesting symptoms over months to years. Typically, biofilm forms at the interface between the implant and its surrounding fibrous capsule and adjacent tissues. The biofilm-host immune response commonly results in a low-grade or fibrotic inflammatory process, with minimal or no suppuration or sepsis [19, 22]. This inflammatory process is irritative or destructive to adjacent tissues and can diminish the long-term stability of the implant and/or capsule. Biofilm infection of orthopaedic implants, such as titanium knee and hip prosthesis can result in chronic osteomyelitis of the adjacent bone, low-grade joint effusions, joint sinus formation and ultimately

delayed loosening and failure of implant [26]. Similarly, biofilm infection of breast implants results in chronic inflammation of the periprosthetic capsule (Fig.3), late seroma formation and ultimately stiffening and deformity of the capsule (capsular contracture) and breast deformity [22].

## Bacterial isolation and diagnosis

Microbiologic diagnosis of biofilm infections is challenging and frequently inaccurate as the sessile bacteria within the biofilm do not grow using standard diagnostic microbiological techniques developed for planktonic bacteria [16]. Hence, many clinicians are of the incorrect opinion that biofilm is not a problem in nonsupparative mesh complications because routine cultures are negative for bacterial growth.

There are multiple techniques to visualise biofilms and identify the resident bacterial species, however these methods are mainly used in research and there is a lack of reliable diagnostic tools for clinical practice. In research, biofilms are detected using a twofold process of direct visualisation and bacterial identification, including (but not limited to the following) [16, 27]

Microscopy:

- Scanning electron microscopy.
- Immunofluorescence.
- Fluorescence in situ hybridisation combined with confocal laser scanning microscopy.
- Scanning laser optical tomography.

Bacterial identification:

- Sonication (to release bacteria from the biofilm matrix) and subsequent standard bacterial culture and identification.
- Molecular diagnosis, e.g. with 16S RNA PCR, transcriptome analysis and comparative genomics or flow cytometry.
- This can enable bacterial identification of one or more organisms and quantification of these bacteria.

## Biofilm and mesh hernia repair

Currently, there are only a few case reports of biofilm identified on hernia mesh in the literature, hence, there is limited clinical evidence linking biofilm infection and mesh complications or hernia repair failure [6, 28]. It could be expected that if biofilm was a cause of mesh infection and other complications, the clinical manifestations may be similar to other surgical implants, such as orthopaedic and breast implants [29]. Hence, these complications may include late low grade localised sepsis, swelling, erythema, late seroma formation, fistula formation, and ultimately mesh-capsule deformity and mesh failure. These complications are uncommon but have been documented in patients with failure of their mesh hernia repairs. However, to date direct correlation and a causal relationship between mesh-hernia complications and biofilm infection has not been demonstrated.

## Effective porosity, mesh construct and material composition

Porosity is a unique property of hernia mesh where by the woven mesh construct with open pores results in tissue ingrowth and mesh–tissue integration [30]. Recent research has shown that pore sizes of over 1000 µm (macroporous) result in less host inflammatory reaction and less fibrosis [31, 32]. In turn this produces less scar plate formation and improved mesh integration, resulting in a superior repair.

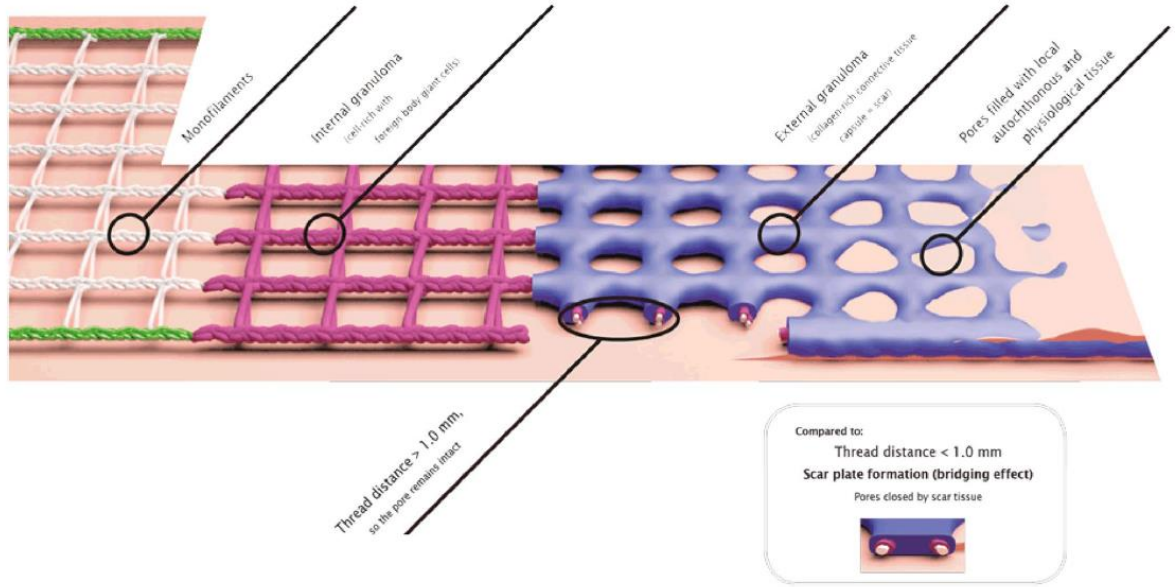


**Fig. 4** Textile porosity refers to the permeable component of a mesh before the body has reacted to the implant. Effective porosity refers to the permeable component of a mesh after the body has reacted to the implant. Rule of thumb: A "pore" less than 1 mm in diameter is closed by the body with scar tissue = 0% effective porosity

Recently, Klinge and Klosterhalfen have reported on a review of over 600 explanted meshes and describe the new concept of ‘effective porosity’ [32](Fig. 4). In this paper the authors argue that under certain circumstances the functional mesh pore size can be reduced. This in turn decreases the effective porosity, negatively impacting on the mesh–tissue integration, the stability of the mesh-hernia repair and promoting fibrosis with scar plate formation. One potential cause for reduction in effective porosity is surgical technique with folding, bending or overstretching of the mesh. Another is axial loading. Axial loading is the result of the forces acting on the mesh after implantation and its effects are dependent on how the mesh is placed (surgical technique) and inherent qualities of mesh polymer composition. Axial load can unduly stretch the mesh construct and may significantly distort the mesh pore size, decreasing effective porosity [6, 32] Fig. 5.

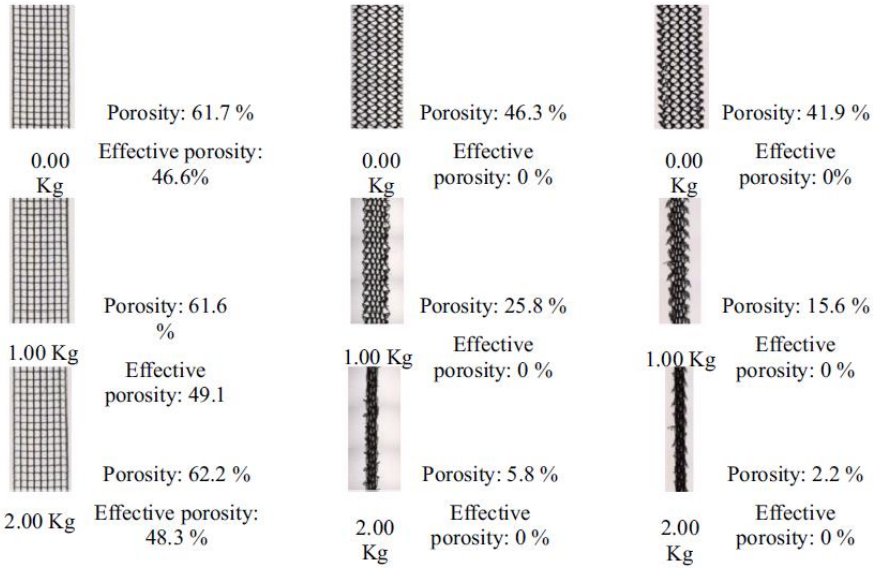


**Fig. 5** Change in pore size (effective porosity) of mesh under axial loading figure supplied by Klosterhalfen et al. [32]

Another factor proposed to reduce effective porosity is the presence of mesh adjuncts such as barrier protection and hybrid meshes. Chemical barriers and/or mode of application (such as single versus double sided, woven attachment versus dipped or coated) may further decrease effective porosity [32]. Hence, the majority of meshes which have adequate porosity at the time of manufacturing may have inadequate effective porosity after implantation due to mesh composition, surgical technique and axial loading [32].

## Bacterial biofilm and effective porosity

We hypothesise that biofilms in and around mesh may be less problematic than in other polypropylene or silicone prosthesis due to the presence of pores within the

mesh. These pores enable tissue–mesh integration and that this may decrease the total continuous surface area available for bacterial adhesions and biofilm development. This mesh–tissue integration may improve immune system interaction that may decrease mesh–bacteria interaction reducing the risk of biofilm formation.

However, if the effective porosity is reduced (due to mesh construct, surgical technique or axial loading), and results in decreased mesh tissue integration, then contamination with biofilm formation may become significant and problematic. This biofilm may further reduce the effective porosity by blocking the remaining pores with their exopolysaccharide coat. This may potentially set up a positive feedback situation with eventual mesh failure. Hence, even with wide pore meshes the combination of reduction of effective porosity and biofilm may result in a chronic inflammatory reaction, similar to that observed with microporous meshes, with bridging fibrosis, mesh contracture, seroma and possible fistula formation. Further, these bacteria within the biofilm may survive for years in this state and potentially result in late mesh infections especially if the patient becomes immunosuppressed following an illness.

## Strategies for prevention of biofilm formation and maintaining effective porosity.

Decreased effective porosity and bacterial contamination with biofilm formation may individually and collectively impact on the mesh–tissue integration and long-term strength and reliability of the mesh-hernia repair. Managing and preventing these complications will require addressing both problems. Mesh design and biomaterials may significantly improve effective porosity [31, 32]. Klinge and Klosterhalfen are advocating meshes which are flat with large and effective pores [32]. Novel, more stable biomaterials such as polyvinylidene difluoride (PVDF) may be more advantageous, compared to polyester meshes that degenerate with time. The role of barrier and coated mesh in reducing effective porosity requires further investigation and may result in moving mesh away from the intraperitoneal position and advocating a retrorectus or preperitoneal approach instead. Improved surgical technique may assist in maintaining the effective porosity by avoiding folding or tension on the mesh at time of insertion.

In studies on orthopaedic and dental implants [29], there is preliminary evidence that titanium may exhibit a partially strong adherence of biofilm forming bacteria, however there is a paucity of scientific data on titanium coated hernia meshes and the risk of biofilm formation. Future studies will need to address issues relating to the coating with titanium in mesh-hernia repair.

Prevention of bacterial biofilm infection remains an important and complex problem and is a focus of ongoing research and evaluation in the orthopaedic and plastic surgical literature. The mainstay of all the recommendations in this body of literature is based on prevention of contamination of the implant and surgical bed with the skin/skin flora at the time of insertion.

As such both specialties have developed “no-touch” techniques and protocols that recommend induction antibiotics directed to skin bacteria to sterilise the surgical field, skin preparation and barrier coverings to “exclude” skin contact. Other measures include haemostasis of implant bed to decrease risk of haematoma as blood is a good food source to grow bacteria, change of gloves between surgical bed preparation and contact with implant, change of instruments where appropriate, use of biostatic or antibiotic implant wash prior to insertion and other devices to insert implants without skin contact.

Additional specialised recommendations include measures such as high-flow/reverse flow circulation to flush bacteria away from the operative table and ventilated “Space-suits” for orthopaedic joint replacement theatre surgeons and scrub staff to prevent contamination from staff skin, hair and clothes. There is increasing clinical and pre-clinical data from these surgical specialties to demonstrate a significant decrease in bacterial contamination is correlated with decreased complications associated with these implants and biofilm infection.

Currently there is extensive development of other biomaterials and implant adjuncts to prevent bacterial contamination and biofilm formation however to date very few if any of these products/adjuncts have made it to the commercial market as safety and efficacy still need to be determined for most these proposed products.

In a recent review Guillame et al. [12, 13] and others advocate a limited number of in vitro and in vivo models to bench test new antiinfective meshes to allow for better comparability in mesh developments.

Currently there is no anti-biofilm product/adjuncts available commercially for hernia mesh. As such the recommendations detailed in this article align with the currently accepted recommendations in both the Orthopaedic and Plastic implant-biofilm prevention literature.

Future innovation may include antibiotic-impregnated mesh or cold plasma wash. Antibiotic-impregnated mesh may prevent bacterial contamination or biofilm formation. Cold plasma wash at time of insertion sterilises mesh by disrupting bacterial cell membrane without affecting human cell membranes.

Belyanski and Heniford et al [33] studied the effect of Lysostaphin impregnation to combat S. aureus infection of porcine grafts. Their study demonstrated a reduction in graft infection highlighting the possible use of antibiotic impregnation as a protection against mesh infection and possible biofilm prevention.

Innovative treatments to prevent and/or treat mesh biofilms are currently under investigation, including smart drug delivery systems with prolonged release kinetics of antibacterial compounds tailored for localised use in hernia repair.

## Conclusion

Late mesh complications are uncommon but result in significant morbidity. These complications are frequently multifactorial involving patient, surgical and mesh-related factors. The dual concepts of effective porosity and biofilm may be important considerations in mesh-related morbidity and should be investigated further. Changing surgical techniques and developing new meshes to maintain effective porosity and reduce biofilm formation may help surgeons to reduce mesh-related complications.

Issues pertaining to effective porosity and biofilm are the subject of research now in progress. This includes research to establish the magnitude of the problem by analysing for biofilm in explanted mesh, removed for non-suppurative reasons. In addition, a porcine model has been developed to implant meshes of different compositions and subsequently analysing these meshes for biofilm, to investigate the potential of different meshes promoting biofilm formation.

The effects of biofilm and inadequate effective porosity may result in mainly long-term consequences such as seroma, late mesh failure (central mesh failure, migration, contracture) and late infection. It is important that all patients undergoing hernia repair are followed up utilising clinical quality registries that combine both patient outcome and all aspects of mesh data, to be able to study and evaluate the magnitude of this issue.

## Acknowledgements

The Microscopy Department, School of Biological Sciences, Macquarie University is kindly acknowledged for providing expertise in electron microscopy. KR is recipient of a CJ Martin Biomedical Early Career Fellowship (#1163634) by the National Health and Medical Research Council, Australia. Chris Hensman is a consultant for Medtronic. Bernd Klosterhalfen is a consultant to FEG Textiltechnik and also an expert witness in the US and Australia in mesh litigation cases.

## Compliance with ethical standards

### Conflict of interest

The authors declare that there is no conflict of interest directly related to this article.

### Ethical approval

Due to the fact that this research uses secondary data (use of data initially collected for anoither purpose)there is no form of identifier or data linkage or results that could generate identifiable information the article was exempt from Ethics approval.

### Human and animal rights

This article does not contain any studies with human participants or animals performed by any of the authors.

## Informed Consent

For retrospective review, formal consent is not required.

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Chapter 5: Statement of Authorship and Publication 4:

# Statement of Authorship

| | |
|---|---|
| Title of Paper | The role of bacterial biofilms in late hernia mesh complications. |
| Publication Status | Unpublished and Unsubmitted work written in manuscript style. |
| Publication Details | N/A |

## Principal Author

| | | | |
|---|---|---|---|
| Name of Principal Author (Candidate) | Paul Patiniott | | |
| Contribution to the Paper | Conceptualisation, Literature Review, Principal Investigator, Data Collection, Data Analysis, Authorship, Review | | |
| Overall percentage (%) | 75% | | |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. | | |
| Signature | | Date | 13/10/2021 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

iv.

| | | | |
|---|---|---|---|
| Name of Co-Author | Anita Jacombs | | |
| Contribution to the Paper | Co-investigator, Co-authorship, Data collection, Data Analysis, Review | | |
| Signature |  | Date | 13/10/2021 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Laurine Kaul | | |
| Contribution to the Paper | Co-investigator, Co-authorship, Data collection, Data Analysis, Review | | |
| Signature |  | Date | 13.10.2021 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Katharina Richter | | |
| Contribution to the Paper | Co-investigator, Co-authorship, Data collection, Data Analysis, Review | | |
| Signature | | Date | 13/10/2021 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Bernd Klosterhalfen | | |
| Contribution to the Paper | Co-authorship, Review | | |
| Signature | | Date | 10/26/2021 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Alex Karatassas | | |
| Contribution to the Paper | Conceptualisation, Supervision and Review | | |
| Signature | | Date | 12/10/21 |

| | | | |
|---|---|---|---|
| Name of Co-Author | Guy Maddern | | |
| Contribution to the Paper | Conceptualisation, Supervision and Review | | |
| Signature | | Date | 13/10/21 |

# Introduction

The impact of bacterial biofilms and effective mesh porosity on hernia repair are unstudied topics to date. Having major implications on many non-hernia disease conditions, bacterial biofilm infection and its possible reduction of mesh porosity and subsequent limitation of mesh-tissue integration may present a plausible explanation for delayed “mesh infection”, chronic seroma, chronic pain, and failure of hernia repair.[1]

Biofilm has been defined as an “aggregate of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface (IUPAC)[2]. Bacterial biofilms are formed when bacteria convert from their planktonic (“free-floating”) genetic phenotype to a sessile genetic phenotype[3]. This enables the bacteria to form communities composed of single or multiple species of microorganisms attached to surfaces that interact with each other and their environment conferring a significant survival advantage[4].

Once the biofilm producing phenotype has been activated the bacteria begin to produce a protein-polysaccharide rich matrix of EPS which encloses them[3,5]. This three-dimensional biofilm matrix provides the resident bacteria a 3-dimentional structure that provides mechanical stability and physical protection from external stressors. The EPS matrix can prevent or limit the activity of immune cells and therapeutic compounds entering or diffusing through the matrix, hence providing a protected haven for bacteria to survive and reproduce[5]. Conversely, the EPS matrix facilitates bacterial communication, important for reproduction and survival, and promotes exchange of genetic information, including antibiotic resistance genes[5,6]. Hence new antibiotic-resistance genes often rapidly spread though bacteria within biofilm[6]. In some cases antibiotic resistance can be increased a thousandfold[7]. Together the physical barrier and swift integration of novel antibiotic-resistance genes makes the eradication of entire biofilm challenging even with best medical interventions[3,5,8].

Device-related infections were the first clinical infections to be identified as having a biofilm aetiology and show that biofilm formation can be facilitated by the host inflammatory response because host inflammatory molecules facilitate adhesion to

the surface of the device[9]. Biofilm formation on medical implants has even led to the characterization of a new infectious disease called chronic polymer-associated infection[10,11]

Skin flora, including *Staphylococcus aureus* and skin commensal coagulase-negative Staphylococci species, in particular *Staphylococcus epidermidis*, are frequently linked to biofilm in orthopaedic joint prostheses and breast implants[12-14]. Implant contamination has been shown to occur mainly at time of insertion, when the risk of implant contact with patient's own skin is highest[12,13]. It is therefore reasonable to expect the above commensal organisms to also be present in hernia mesh related biofilm, accordingly this consideration was factored into our study design.

In this study our main objective was to investigate whether there was evidence of bacterial biofilm on mesh used in inguinal hernia repairs that had been explanted due to complications including recurrence and chronic pain.

# Methods

## Confocal microscopy with immunofluorescence

Thirty deidentified paraffin-embedded tissue sections from explanted groin hernia mesh in patients with chronic pain, were supplied to the research team courtesy of Professor Bernd Klosterhalfen, Aachen, Germany.

Each specimen was sectioned into duplicate into 5 µm slices using a microtome and placed on a negatively charged microscopy slides. The specimens were heated to $600^{O}$ C in a heat bath, deparaffinised in xylene and rehydrated in serial ethanol and phosphate-buffered saline before fixation. Each mesh specimen was stained with a peptide nucleic acid fluorescence in situ hybridization (PNA FISH) probes from AdvanDx (OpGen® Denmark) specific for *Staphylococcus aureus* and *Staphylococcus epidermidis* rRNA using the manufacturer's protocol.

The slides were mounted and imaged with an Olympus FV3000 Laser Scanning Confocal Microscope for analysis and detection of bacterial biofilm in the explanted hernia mesh-tissue. The resulting images were independently reviewed by

consultant clinical microbiologist and the results were compared and found to be similar. The findings were subsequently correlated with the clinical and mesh information.

## DNA extraction from FFPE tissue and qPCR

A thin slice of mesh FFPE tissue was cut with a sterile razor blade and deparaffinated using xylene and ethanol. Paraffin cleaned tissue was digested in 1 mg/ml proteinase K at 56°C for 2 hrs or overnight (until the sample has been completely lysed). Followed by 0.5 mg/ml lysozyme at 56°C for one hour and 0.5 mg/ml proteinase K at 56°C for an additional hour. The cell lysate was incubated at 90°C for one hour to break the cross-link of DNA. Then DNA was extracted using High Pure FFPET DNA isolation kit (Roche, product 06650767001) according to the manufacturer instruction.

Quantitative real-time PCR (qPCR) targeting 16S rRNA genes was performed to determine the total number of bacteria using universal eubacterial primer 341F 5'-CCTACGGGAGGCAGCAG-3' and 534R 5'-ATTACCGCGGCTGCTGG-3' to amplify a 194 bp amplicon in 16s rRNA gene. The extracted DNA was normalised to human 18s rRNA gene using primer pair 756F 5′-GGTGGTGCCCTTCCGTCA-3′ and 877R 5′-CGATGCGGCGGCGTTATT-3′ to amplify a 122 bp amplicon in 18s rRNA gene.

*Staphylococcus* Spp. was detected using *Staphylococcus* genus specific primers 704F 5'-GGCGAAGGCGRCTTTCTGG-3' and 798R 5'-CGTTTACGGCGTGGACT-3' to amplify a 95 bp amplicon in16s rRNA gene. *Staphylococcus aureus* was detected using *S. aureus* species specific primers 5'-GCGATTGATGGTGATACGGTT-3' and 5'-AGCCAAGCCTTGACGAACTAAAGC-3'targeting the *nuc* gene (Brakstad et al 1992).

20 µl qPCR reaction containing 1X PowerUp SYBR Master Mix (Applied Biosystems, Cat# A25741), 400 nM forward and reverse primers and 100 ng DNA template was processed under following condition in the ViiA™ 7 Real-time PCR System (Applied Biosystems): an initial temperature of 50°C for 2 minutes followed by 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A known concentration of S. aureus genomic DNA and human DNA (102–108 copies/µl) was used to produce standard curve in each qPCR reaction. A no template control

containing nuclease-free water instead of DNA template was also included in the PCR.

### Ethical approval

This study was reviewed by the Secretariat, Human Research Ethics Committee and was deemed to meet the requirements of the National Statement on Ethical Conduct in Human Research 2007 (Updated 2018). According to provisions within the National Statement, The University of Adelaide classifies research that carries only negligible risk and involves the use of existing data that contains only non-identifiable data about human beings, to be exempt from ethical review. The research conducted with these materials only, as part of this project meets these requirements and has been authorised as exempt from requiring ethical review.

## Results

This is a retrospective observational study of 20 explanted mesh tissue specimens that were analysed for evidence of *S. aureus* and *S. epidermidis* biofilm infection with microscopy and DNA identification. Each specimen was an explanted polypropylene-based mesh, from various manufacturers, which had previously been inserted as part of an open or laparoscopic inguinal hernia repairs or incisional hernia repair.

The majority of patients were male (n=18; 95.0%) with a median age of 50 years and 6 months. Most of the hernia repairs were done with an open-Lichtenstein technique (n=13; 65.0%), laparopscopic (TEPP or TAPP) hernia repair (n=4; 20.0%), Plug mesh repairs (n=2; 10.0%) and open incisional hernia sublay repair (n=1; 5.0%). The median time from hernia repair to explantation was 24.5 months (range 8 to 72 months) and there were a range of polypropylene mesh products explanted as detailed in Table 1. The all patients had one or more indications for explanation including chronic pain (n=14; 70.0%), hernia recurrence (n=9; 45.0%) and mesh shrinkage (n=4; 20.0%). Two patients (10.0%) had both hernia recurrence and mesh shrinkage or both hernia recurrence and chronic pain. One patient (5.0%) had both chronic pain and mesh shrinkage and one patient (5.0%) had hernia recurrence, chronic pain and mesh shrinkage. No patient had documented infective symptoms.

## Bacterial Biofilm Identification

Each explanted mesh was assessed for presence of bacteria with two techniques confocal microscopy for bacteria specific biofilm and DNA-PCR bacterial identification for 2 of the most common bacteria involved in implant biofilm infection *S. aureus* and/or *S. epidermidis*.

## Confocal Microscopy

Fifteen (75.0%) mesh sampled demonstrated confocal microscopic evidence of bacterial biofilm attached to the surface of the mesh. Three (15.0%) mesh each were positive for *S. aureus* only or *S. epidermidis* only and nine (45.0%) were positive for both bacteria FIGURES.

## PCR Identification

PCR Bacterial biofilm identification was positive in 17 (85.0%) mesh samples. Seven (35.0%) mesh samples were positive for *S. epidermidis* only, 2 (10.0%) were positive for *S. aureus* and 8 (40.0%) were positive for both bacteria. When both techniques were combined there was concordance with both confocal and PCR-bacterial biofilm identification positive for one or both bacterial species in 13 (65.0%) mesh samples. Bacterial biofilm were identified by either confocal or PCR identification in 6 (30.0%) of mesh samples and only 1 (5.0%) mesh sample was negative from bacterial biofilm by both techniques.

# Tables

Table 1 Excised Mesh Products

| **Product** | **Number (%)** |
|---|---|
| Ultrapro | 7 (35.0%) |
| Prolene/Soft Prolene | 4 (20.0%) |
| Optiline | 2 (10.0%) |
| Perfex Plug | 2 (10.0%) |
| Vyprol | 2 (10.0%) |
| Adhesix | 1 ( 5.0%) |
| Atrium | 1 ( 5.0%) |
| TiMesh | 1 ( 5.0%) |

Table 2 Concordance of Bacterial Biofilm Identification by Confocal Microscopy and PCR-Bacterial Identification on Hernia Mesh

| **Bacterial Identification** | **Number (%)** |
|---|---|
| Imaging & PCR Positive | 13 (65.0%) |
| Imaging or PCR Positive | 6 (30.0%) |
| Imaging & PCR Negative | 1 ( 5.0%) |

# Adelaide Group – Select Figures (Confocal Microscopy / Immunofluorescence)

Immunofluorescent probes: Green – Staph Aureus, Red – Staph Epidermidis

Specimens provided by Professor Bernd Klosterhalfen, Slide Preparation & Microscopy Conducted by Laurine Kaul, Katarina Richter, Anita Jacombs and Paul Patiniott, Microbiological Analysis and Input by Dr Morgyn Warner.

Figure 1: DAPI 60x merge – Demonstrating presence of *Staphylococcus aureus* biofilm colonies (Green staining of individual bacterial cells) nestled in and around the mesh (blue) the tissue of the peri-mesh fibrous capsule.

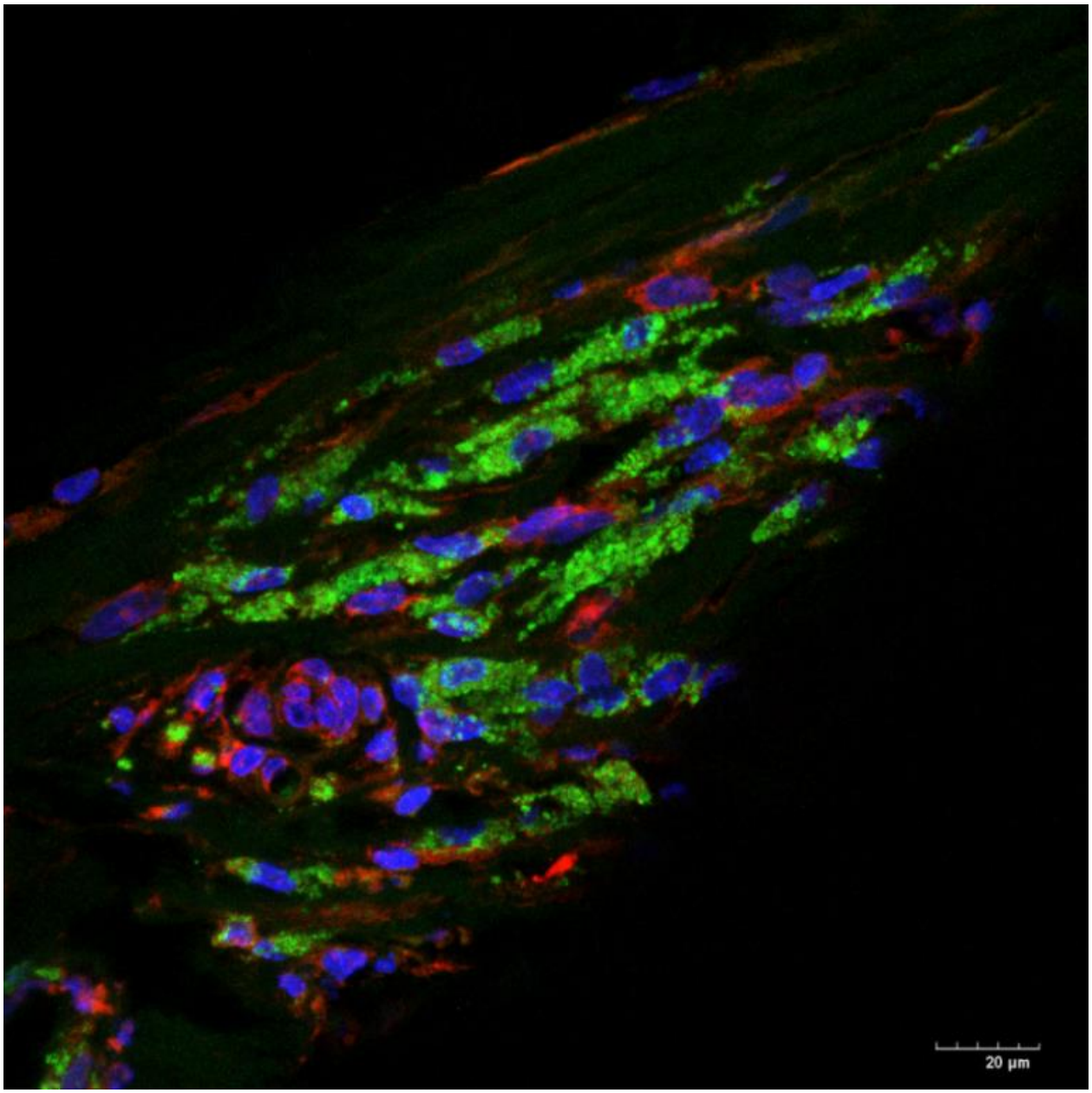


Fig 2: DAPI 60x z-Stack from inguinal mesh explanted for chronic pain. All 4 images are of the same section showing: A) background tissue before bacterial identification, the blue strand is a mesh fibre, B) tissue with staining for green *Staphylococcus aureus* bacterial biofilm, C) tissue with staining for red *Staphylococcus epidermidis* biofilm and D) merged image identifying both *S. aureus* and *S. epidermidis* biofilm

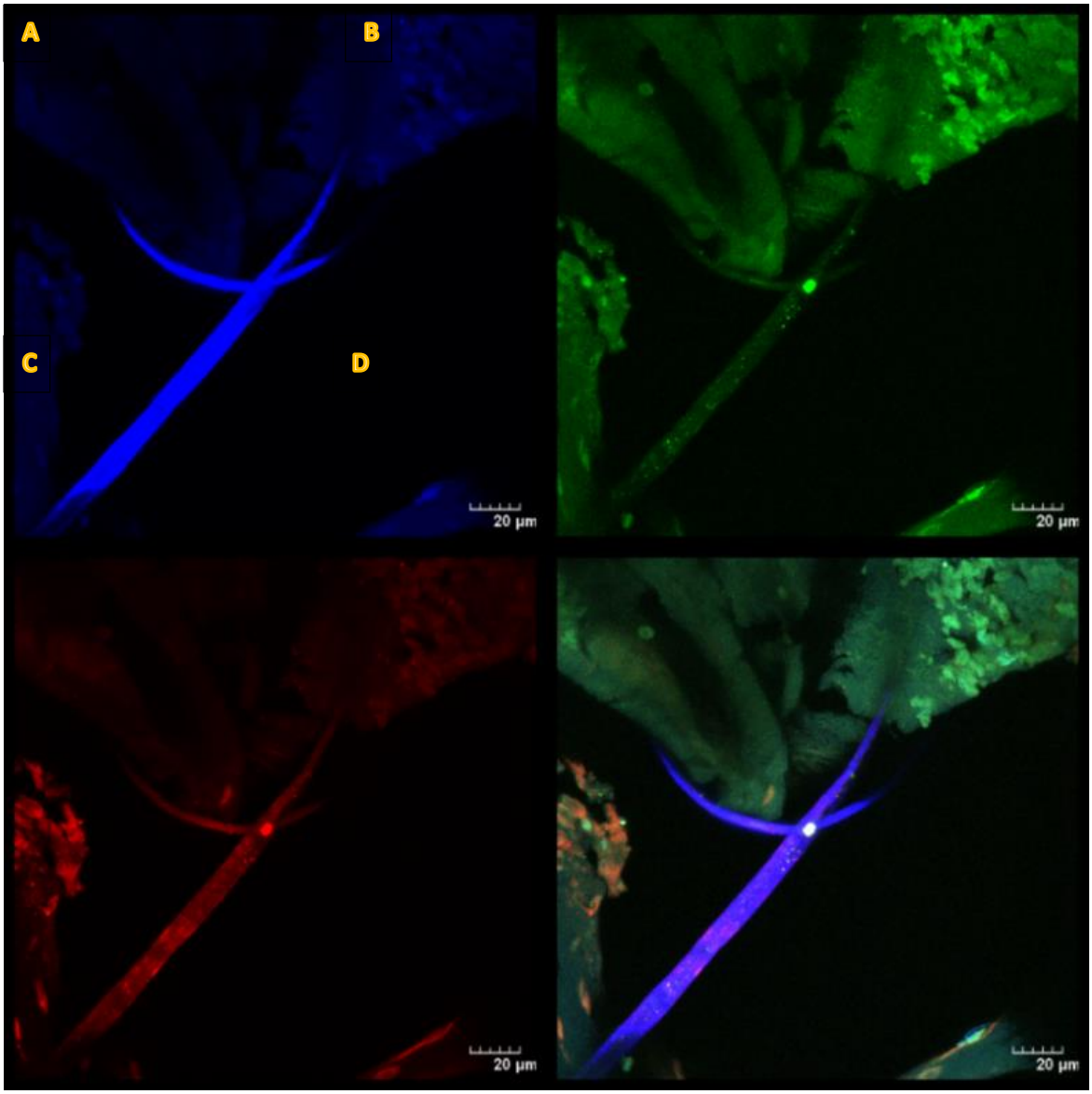


Fig 3: DAPI 60x z-Stack A) background tissue before bacterial identification, B) tissue with staining for green *Staphylococcus aureus* bacterial biofilm, C) tissue with staining for red *Staphylococcus epidermidis* biofilm and D) merged image identifying florid infection of both *S. aureus* and *S. epidermidis* biofilm

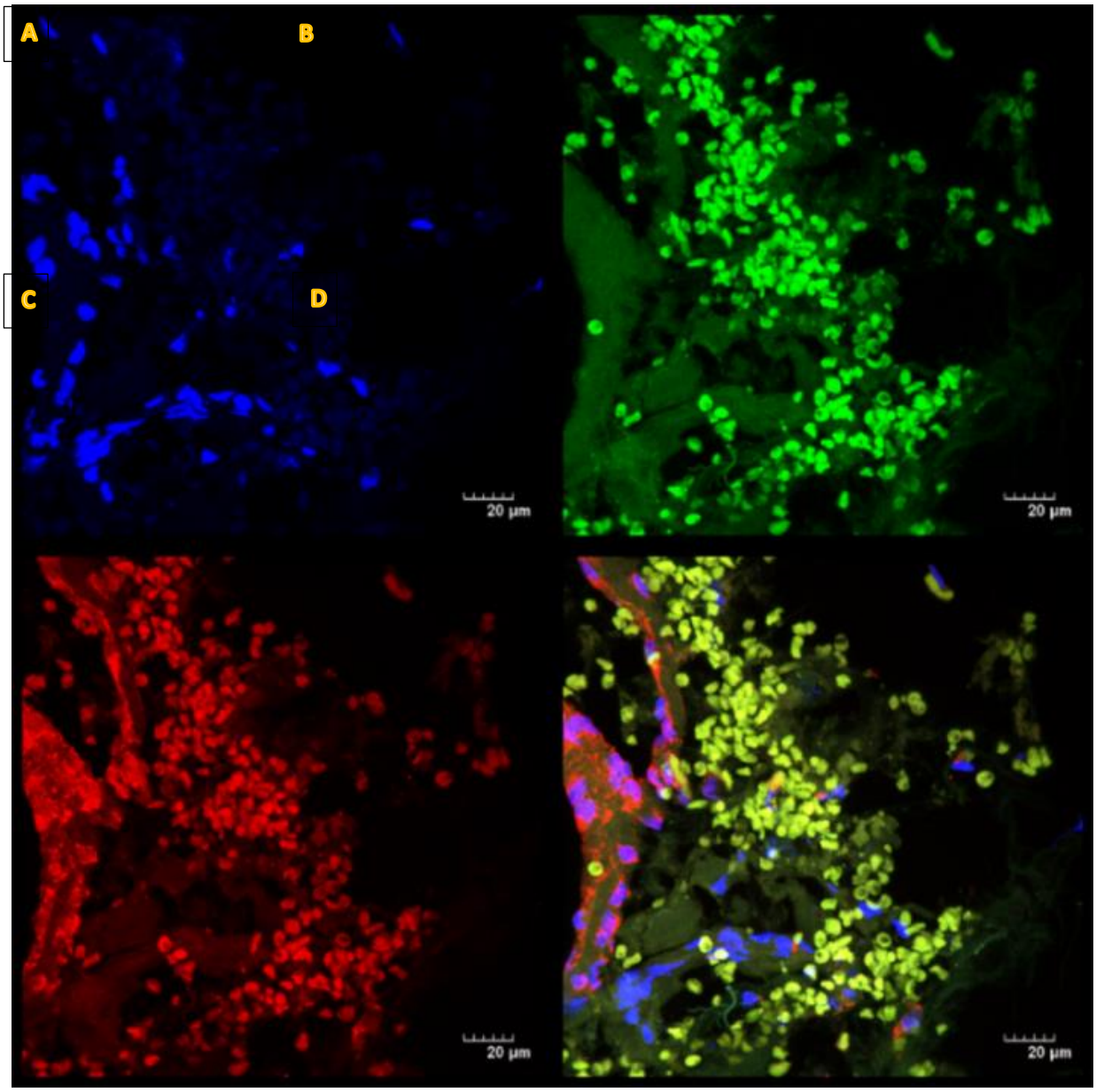


Fig 4: 60x DAPI z-Stack demonstrating both *S. aureus* bacteria (green) and *S. epidermidis* bacteria (red) embedded in polymicrobial community contained within mucilage (EPS) consistent with biofilm.

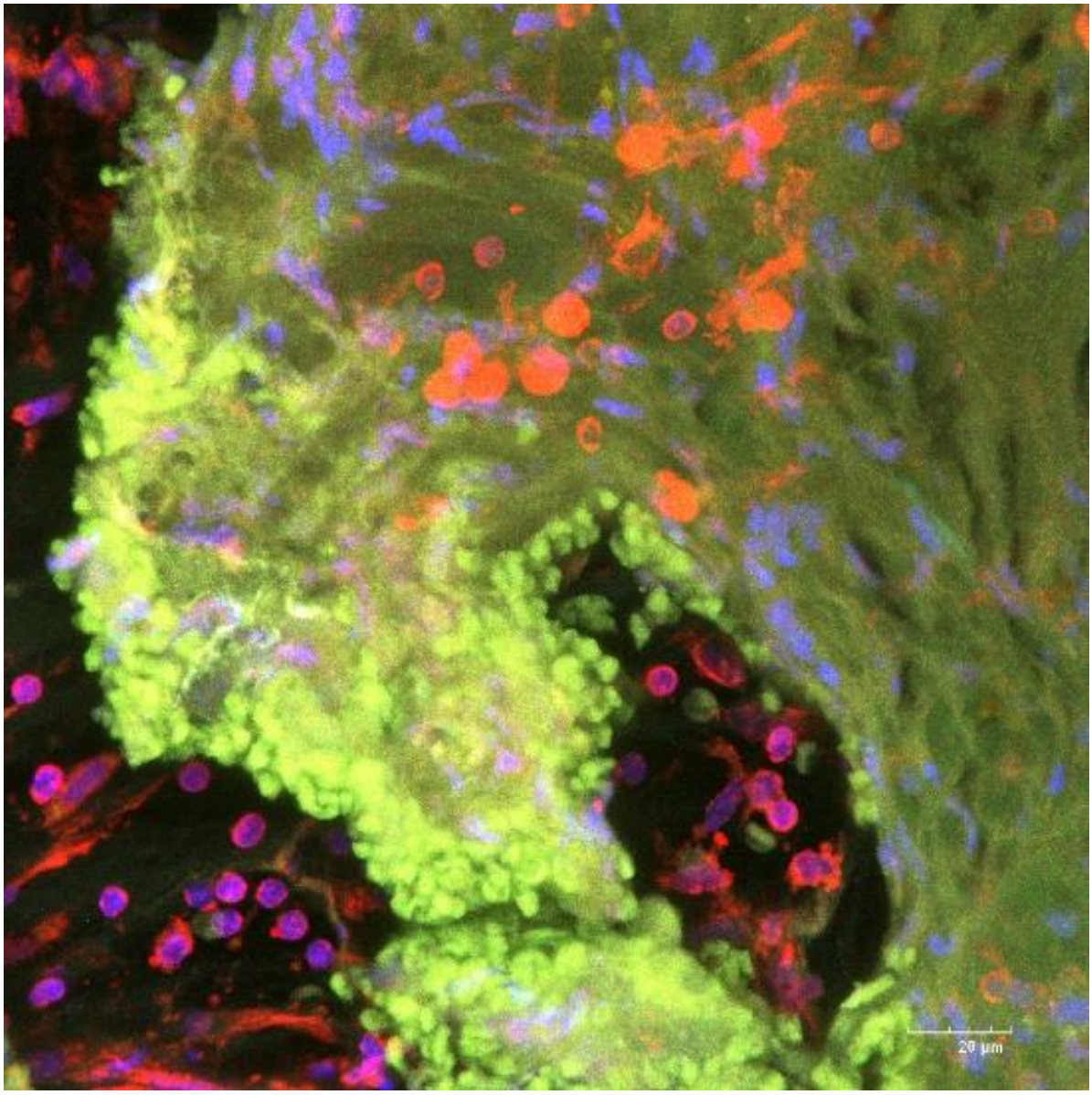


Fig 5: Explanted from 49y.o. Female – Inguinal Hernia – UltraPro – Polypropylene – 38 Months – Indication for removal: Chronic Pain Mesh Specimen  20x confocal magnification DAPI merge – **BLUE** nuclei within tissue cells.

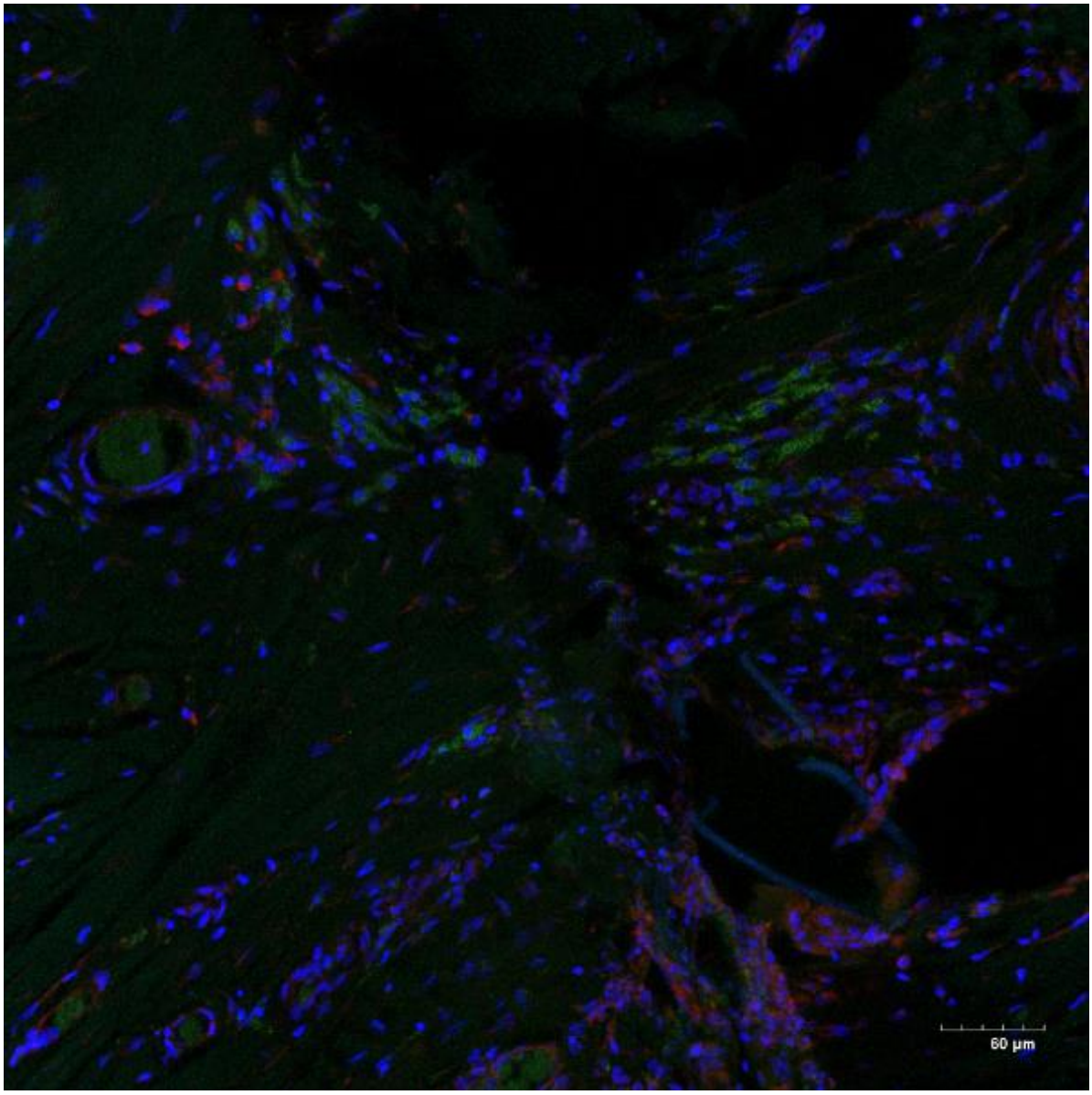


Fig 6: Explanted from 76 y.o. male – TiMesh – Polypropylene – Inguinal Hernia – 12 months – Indication for removal: Chronic Pain - DAPI 20x – Z-stack – Note the immunofluorescent **GREEN** Staph. Aureus biofilm mucilage aggregation towards the centre of the image.

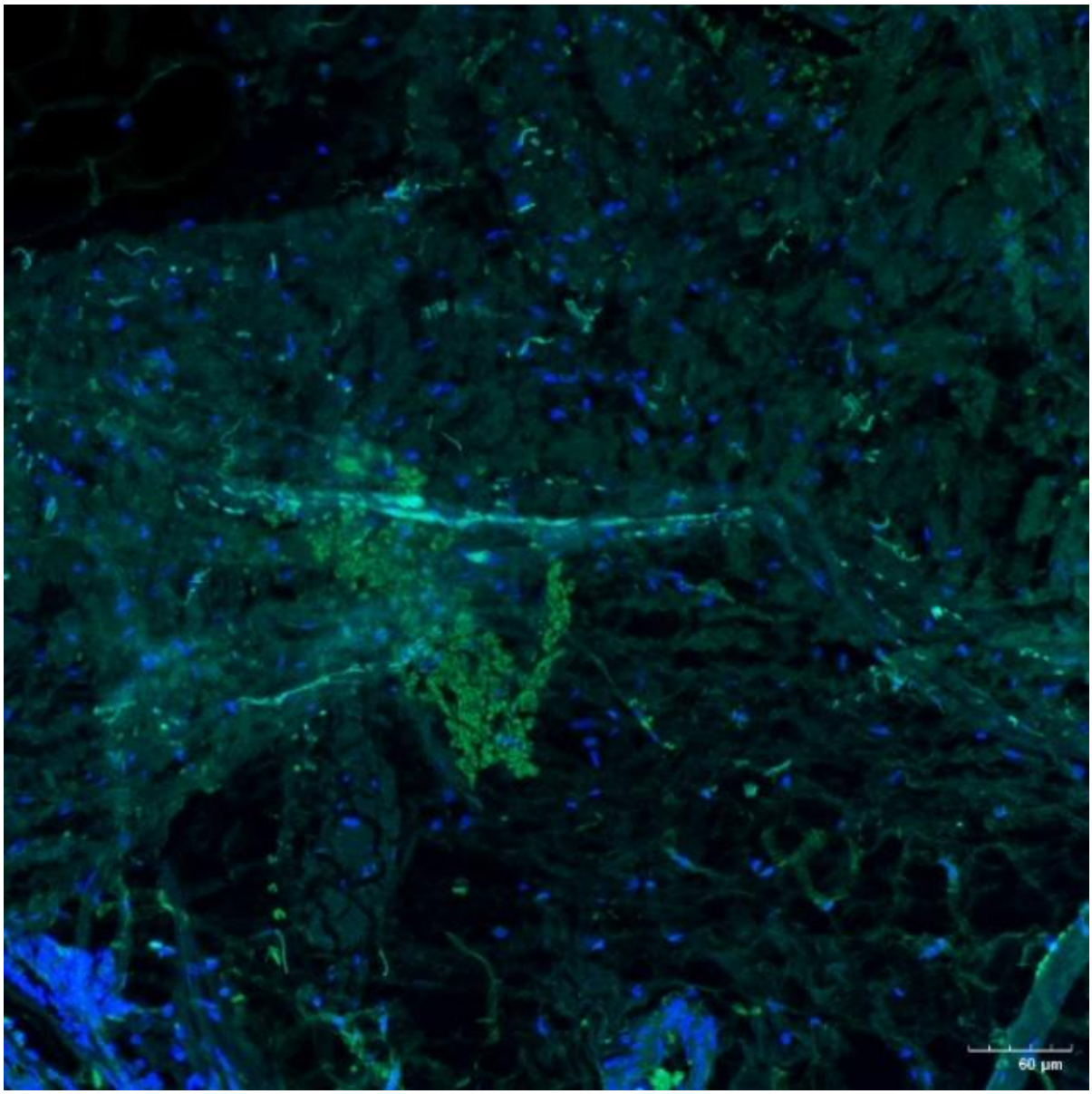
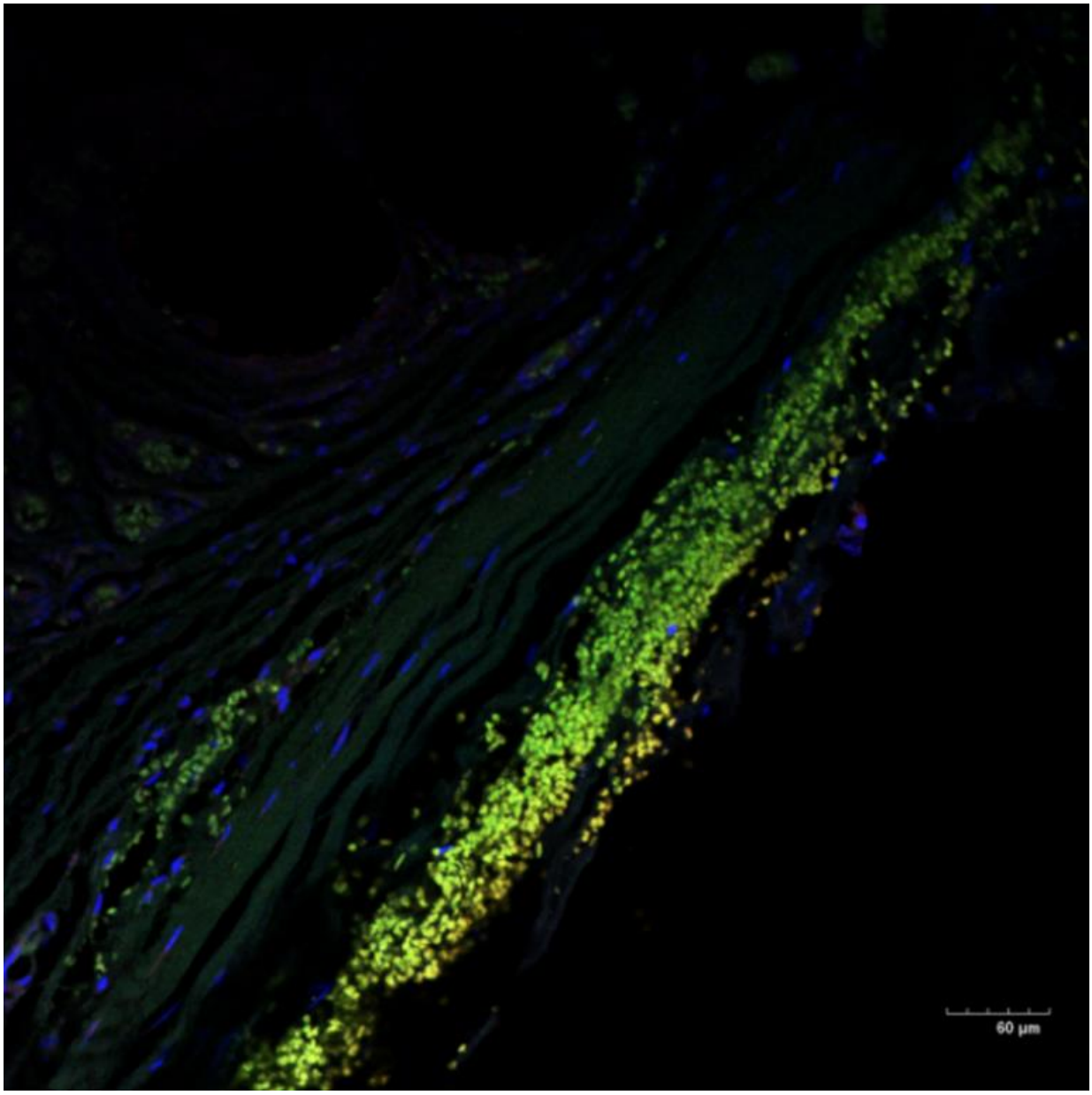


Fig 7: Explanted from 44y.o. male – Optilene – Polypropylene Mesh – Inguinal – 16 months - Indication for explantation: Chronic Pain AND Shrinkage Mesh Specimen 4099  20x DAPI - Consistent with biofilm adjacent to tissue.



## Discussion

Our study set out to investigate the role of biofilms in a variety of chronic mesh complications as described above in the results. The study protocol was designed based on established techniques for the investigation of bacterial biofilms.

We discovered evidence on confocal microscopy of bacterial biofilm, identified in the significant majority of specimens examined in this series. *S. aureus* and *S. epidermidis,* skin flora that commonly cause biofilm formation on surgical implants, have been identified on fixed mesh explant samples for common mesh complications such as pain, recurrence and shrinkage. These results suggest that bacterial biofilm readily infect surgical mesh and may be implicated to common mesh complications. Further research is required to investigate the causal relationship between mesh-biofilm infection and how it affects the integrity of surgically inserted mesh and its complications.

The pervasiveness of biofilm involvement was unexpected and represents an unique insight into the importance of biofilm in the pathophysiology of chronic mesh complication and failure, this is a concept that to date appears to be relatively unexplored in the literature, in contrast to the plethora of publications relating to biofilms in other medical devices.

A major strength of this study was that the research question necessitated multidisciplinary collaboration of investigators including surgeons, scientists, microscopists, microbiologists and pathologists. However this extensive collaboration perhaps also indirectly contributed to the study's greatest limitation, as the international and interstate transport of the fixed specimens raised the potential for confounders to detrimentally affect accurate diagnosis of biofilm positive implant infections & identification of their bacteria in formalin-fixed specimens. Least of all the increased risk of contamination as well as processing (imaging & PCR) artifact – inhibiting both processes from accurately identifying its target.

A similar study with "non-contaminated" fresh samples would be the more accurate diagnostically, allow additional investigations to be conducted and give a better

understanding of the true burden of biofilm disease in mesh hernia repairs that require revision surgery or mesh explantation.

Mesh has become an essential component of hernia repair, however is not biologically inert as first considered, increased bacterial adherence is a multifactorial result that not only depends on the bacteria but also on the textile, physicochemical properties and composition as well[15]. The interaction between the biofilm pathogen and the host inflammatory response is complex and involves an alteration of the host environment[9]. Biofilm formation can be facilitated by the host inflammatory response because host inflammatory molecules facilitate adhesion to the surface of the device, particularly with staphylococci[10,16].

In 2012 Klinge and Klosterhalfen made a critical distinction between simple mesh porosity – the percentage area of mesh which is not covered by filaments in contrast to the *effective* mesh porosity representing only the area of *"good"* pores where bridging of scar tissue is avoided by sufficient inter-filamentary distance[17]. Furthering this concept, in 2019 Jacombs et. al determined that if the effective porosity is reduced due to mesh construct, surgical technique or axial loading such that it results in decreased mesh tissue integration, then contamination with biofilm formation may become significant and problematic[1]. In a vicious cycle, this increased biofilm formation in turn is likely to further reduce the effective porosity by blocking the remaining pores with the EPS coat.

Our findings, in conjunction to other similar studies, necessitate that General and Hernia Surgeons have a good understanding of bacterial biofilm disease;

1) how it infects surgical implants; 2) the common bacteria involved in these infections; 3) the mechanism involved in biofilm causation of implant failure & complications; 4) understanding how biofilm infections can be prevented which is preferable; 5) developing treatments – currently there is no good treatment other than implant removal.

An improved understanding of this relationship and the relevant underlying pathological mechanisms will underpin the development of biofilm-resistant mesh devices, prophylactic treatments and other important technological advancements to reduce hernia mesh complication rates in patients.

As Surgeons, in advocating for our patients we are obliged to demand that industry considers effective porosity and biofilm prevention in future mesh design. Notwithstanding international media scrutiny and high profile class action litigation relating to surgical mesh device complications and the ensuing political pressure invariably resulting in increased government imposed regulatory requirements of said devices.

This study in conjunction with emerging scientific evidence in the literature is further justifying the utility of a longitudinal hernia mesh registry akin to the established cardiac implant and prosthetic joint registries, bringing hernia surgeons in line with our surgical colleagues from other disciplines.

## Conclusion

As a preliminary study these results suggest that there is presence of biofilm involving common biofilm forming bacteria resulting in clinically relevant infections and surgical implant complications in most samples. Our findings highlight the pervasiveness of biofilm involvement in chronic mesh complications and failure. There is need for further studies on the topic to further characterise the underlying nature of this clinically significant relationship.

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# Chapter 6: Conclusions

Through a systematic review of the literature and an audit of ANZASM data, we found that the risk-adjusted mortality rate for elective abdominal wall hernia surgery in Australia is very low and compares favourably to international statistics. Despite the low absolute numbers, the factors which were most significantly associated with increased perioperative mortality in patients undergoing elective surgical abdominal wall hernia repair included higher age, cardiorespiratory comorbidity, and incisional hernia repair. The findings of this study support adopting a judicious approach when advising this subgroup of patients on the risks and benefits of proceeding with elective abdominal wall hernia repair surgery.

Our investigation into hernia mesh tissue integration (MTI) utilising a porcine model is one of the first to propose a functional, biological standardised model for comparing hernia mesh products. The results are encouraging and demonstrate that this is a robust and transferrable model for assessing MTI in hernia mesh. It is important to emphasise that this is a pilot study providing grounds for progressing to a proof-of-concept MTI index main study which will involve increasing the number of subjects and observing integration over a longer timeframe. Longer term studies will facilitate the development of a degradation index to supplement the integration, fibrosis and adhesion indexes. It is envisaged that the MTI Index will be a useful tool for individualising hernia treatment for patients, the ultimate intention for this model is that it will be utilised synergistically with long term mesh-patient outcome registries and databases to inform improved matching of mesh to patient, particularly in the setting of the complex hernia repair and abdominal wall reconstruction.

There is a paucity of information in the literature on bacterial biofilms and the impact on outcomes following mesh hernia repair. Our findings highlight the pervasiveness of biofilm involvement in chronic mesh complications and failure. Late mesh complications are uncommon but result in significant morbidity. These complications are frequently multifactorial involving patient, surgical and mesh related factors. Changing surgical techniques and developing new meshes to maintain effective porosity and reduce biofilm formation may help surgeons to reduce mesh related complications.

The dual concepts of effective porosity and biofilm may be important considerations in mesh related morbidity and should be investigated further. There is need for further studies on the topic to further characterise the underlying nature of this clinically significant relationship.

Thesis End

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