

Buttressing Staples with Cholecyst-derived Extracellular Matrix (CEM) Reinforces Staple Lines in an *Ex Vivo* Peristaltic Inflation Model.

Krishna Burugapalli, MSc, PhD;¹ Jeffrey C. Y. Chan, MBBCh, MMedSci(Hons), MRCS;^{1,2} John L. Kelly, MBBCh, MD, FRCS(Plast);² Abhay Pandit, MS, MPH, PhD¹

¹National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

²Department of Plastic, Reconstructive and Hand Surgery, University Hospital Galway, Galway, Ireland

Reprint Requests to: Prof. Abhay Pandit, MS, MPH, PhD, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

E-mail: abhay.pandit@nuigalway.ie

ABSTRACT

Background: Staple line leakage and bleeding are the most common problems associated with the use of surgical staplers for gastrointestinal resection and anastomotic procedures. These complications can be reduced by reinforcing the staple-lines with buttressing materials. The current study reports the potential use of cholecyst-derived extracellular matrix (CEM) in non-crosslinked (NCEM) and crosslinked (XCEM) forms, and compared their mechanical performance with clinically available buttress materials (small intestinal submucosa, SIS and bovine pericardium, BP) in an ex vivo small intestine model.

Methods: Three crosslinked CEM variants (XCEM0005, XCEM001 and XCEM0033) with different degree of crosslinking were produced. An ex vivo peristaltic inflation model was established. Porcine small intestine segments were stapled on one end using buttressed or non-buttressed surgical staplers. The opened, non-stapled ends were connected to a peristaltic pump and pressure transducer and sealed. The staple lines were then exposed to increased intraluminal pressure in a peristaltic manner. Both the leak and burst pressures of the test specimens were recorded.

Results: The leak pressures observed for non-crosslinked NCEM (137.8 ± 22.3 mmHg), crosslinked XCEM0005 (109.06 ± 14.14 mmHg), XCEM001 (150.07 ± 15.97 mmHg), XCEM0033 (98.8 ± 10.47 mmHg) reinforced staple lines were significantly higher when compared to non-buttressed control (28.3 ± 10.8 mmHg) and SIS (one and four layers) (62.6 ± 11.8 and 57.6 ± 12.3 mmHg, respectively) buttressed staple lines. NCEM and XCEM were comparable to that observed for BP buttressed staple lines (138.8 ± 3.6 mmHg). Only specimens reinforced staple lines were able to achieve high intraluminal

pressures (ruptured at intestinal mesentery) indicating that buttress reinforcements were able to withstand pressure higher than that of natural tissue (physiological failure).

Conclusions: These findings suggest that the use of CEM and XCEM as buttressing materials is associated with reinforced staple lines and increased leak pressures when compared to non-buttressed staple lines. CEM and XCEM were found to perform comparably with clinically available buttress materials in this ex vivo model.

Key words: Staple line reinforcement, buttress, cholecyst-derived extracellular matrix, ex-vivo, linear stapler

INTRODUCTION

Anastomotic leakage and bleeding at the staple line are devastating complications after gastrointestinal surgery.^{1,2} Stapling devices are commonly used and allow surgeons to perform speedy resection and anastomosis. These devices also allow more complex minimally invasive, laparoscopic procedures to be performed.³⁻⁵ While stapled colorectal anastomoses have not demonstrated reduction in complications,⁶ stapled ileocolic anastomosis is associated with fewer leaks when compared to hand-sutured anastomosis.⁷ Regardless, their use is widespread as tissue handling and operating time can be shortened considerably. Persistent air leakage after lung resection is commonly reported.⁸ More complications are anticipated as these surgical stapling devices are gaining popularity in other specialties including gynaecological,^{9,10} and hepatobiliary¹¹ procedures.

In an effort to reduce leakage and bleeding complications associated with surgical stapling devices, various strategies have been proposed. These strategies include the use of autologous tissue,^{12,13} tissue glue¹⁴ and staple line buttress reinforcement materials. Buttress reinforcement materials are comprised of various synthetic polymers and biologically derived materials. Examples of clinically available buttress reinforcement materials are bovine pericardium (Peri-strips[®]),¹⁵⁻¹⁹ expanded-polytetrafluoroethylene (ePTFE[®]),^{18,20} polyglycolic acid (PGA)-trimethylene carbonate (TMC) copolymer (Gore Seamguard[®])^{21,22} and small intestinal submucosa (Surgisis[®])²³⁻²⁶. These products have demonstrated some success in reducing leakage and bleeding complications associated with staple lines.

In our research facility, a new biomaterial called cholecyst-derived extracellular matrix (CEM) has recently been developed.²⁷⁻³² CEM is composed of decellularized extracellular matrix obtained from the perimuscular subserosal connective tissue of porcine cholecyst (gall bladder) wall.²⁷ Our evaluation has shown that CEM has mesh-like architecture and nano-scale topography. These features are important for supporting cellular functions, tissue ingrowth and vascular infiltration. Specifically, this biomaterial has the ability to support both allogenic²⁹ and xenogenic cells²⁷ in vitro. In addition, the mechanical properties of CEM were shown to be in the physiological range to suit the requirements for soft tissue reinforcement applications.²⁸

The purpose of this study was to explore the possibility of using CEM as staple line buttress reinforcement material. Using an ex vivo porcine small intestine model, this study aimed to investigate the leak pressure and burst pressure of stapled porcine small intestines buttressed with CEM produced in our laboratory. The effect of crosslinking CEM using carbodiimide was also studied to evaluate whether this process would have any beneficial or detrimental effect in this model. Non-buttressed staple lines and two types of clinically available buttress reinforcement materials were used as controls.

METHODS

Materials

All chemical reagents were purchased from Sigma Ireland Ltd. (Dublin, Ireland) unless otherwise stated. Fresh porcine cholecysts and small intestines were obtained from market weight farm-reared pigs (Sean Duffy Exports Ltd., Gort, Ireland) and transported to the laboratory on ice. The intestine specimens (length of 45 to 50 cm) were thoroughly

washed to remove any blood and luminal contents with running water. They were divided to obtain segments of approximately 50 cm in length and kept in phosphate buffered saline (PBS) solution (4°C) with 1% penicillin-streptomycin until testing (<24 hours). Cholecysts were processed and decellularized to obtain CEM according to a standardized method reported earlier.²⁷ Fresh decellularized CEM samples were used for crosslinking. The non-crosslinked CEM (NCEM) samples were freeze dried (Vertis Advantage Freeze Dryer, Gardiner, NY) for storage until further testing. A linear cutting stapler (Ethicon - Proximate-100, Johnson & Johnson Ireland Ltd., Dublin, Ireland) with 100 mm long, 4.8 mm staple-height cartridges was used in this study. This stapling device divides and staples intestine segments by placing two staggered parallel rows of staples on either side of the division (Figure 1).

Buttress materials

Seven different staple line buttress materials (Table 1) were used to reinforce staple lines on the intestine segments using an ex vivo model. Non-crosslinked CEM samples (NCEM) were used as buttress material without further processing. Three different crosslinker concentrations, namely, 0.0005, 0.001 and 0.0033 mmoles of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS) per mg of CEM were used to obtain three variants of crosslinked CEM samples (XCEM0005, XCEM001 and XCEM0033).

In a typical crosslinking process, 0.0005 mmoles of EDC and 0.0005 mmoles of NHS per mg of CEM were used to produce XCEM0005. The numerical suffix following XCEM indicates the crosslinking concentration used. The crosslinking was carried out in 50 ml of 4-morpholinoethane sulfonic acid buffer (MES) (50mM, pH 5.5) for 4 hrs at 37°C with

intermittent shaking. The crosslinked CEM (XCEM) were freeze dried for storage until testing.

One-layer and four-layer small intestinal submucosa specimens (SIS1 and SIS4), (Surgisis[®] and Surgisis[®] ES, Cook, Inc., Bloomington, IN, USA) and glutaraldehyde crosslinked bovine pericardium (BP), (Peri-strips[®], Synovis Surgical Innovations, St. Paul, MN, USA) were also evaluated. Non-buttressed staple lines were used as controls.

Ex vivo testing

Small intestine segments were stapled and divided into two segments of approximately 25 cm using the linear surgical stapler. Buttressed staple lines were created by applying approximately 10 cm x 1 cm strip of buttress test material on each arms of the stapler prior to stapler application. Each of the divided segments had a stapled end (Figure 1) and an opened (non-stapled) end. Two plastic tubes were inserted into the opened lumen and secured water tight using Teflon tapes. One of the tubes was connected to a peristaltic pump (Watson-Marlow 323S, Watson-Marlow, UK), while the second tube was connected to a pressure transducer (ZSE30, SMC Pneumatics Ltd., Saggart, Ireland). Data from the pressure transducer was recorded on a computer using a USB universal input acquisition tool and associated software (myPCLab[™], Audon Electronics, Nottingham, UK) throughout the experiment.

Each stapled intestine segments were inflated with aniline blue solution in a peristaltic manner at a flow rate of 180 rpm with 10 ml volume increments every 10 seconds. The specimens were subjected to increasing intraluminal pressure which was recorded simultaneously using the pressure transducer and the data acquisition unit. The leak pressure was defined as the lowest pressure at which the blue solution leak was observed

(all occurred at the staple lines) and this was recorded. Inflation was continued until tissue or staple line failure. The burst pressure (defined as the pressure when the intestine ruptured or staple line failed) and the site of rupture for each specimen were recorded. Twelve intestinal segments were created for each of the buttress materials and non-buttressed control. In addition, in order to assess the physiological ultimate burst pressure of the intestines specimens, both ends of intestine segments were secured (leak-free) using Teflon tapes and peristaltic inflation was performed until tissue failure.

Statistical analysis

Data were analyzed out using statistical software (SPSS v.14). Statistical variances between groups were determined by one-way analysis of variance (ANOVA). Tukey's test was used for *post hoc* evaluation of differences between groups. A *p* value of <0.05 was considered to be statistically significant. All data represented are expressed as mean \pm standard error (SE) of mean.

RESULTS

All non-buttressed staple lines failed at the staple lines. Leak started at the non-buttressed staples (Figure 2) at an average pressure of about 28.28 ± 10.76 mmHg. An average maximum intraluminal pressure of 78.43 ± 6.25 mmHg was attained and thereafter the intraluminal pressure decreased due to increased leakage from the staple lines. The leakage rate at the non-buttressed staple lines was so high (Figure 3) that none of the intestine segments burst at the mesentery (physiological failure was not reached).

For the buttressed staple lines, the first signs of failure were observed as the blue dye leaked at the staple line for all intestine specimens tested, except for one XCEM001 and

two BP-buttressed staple lines (no leak observed), before the final burst at the mesentery of the intestine segment. Figure 4 shows the leak pressures (intraluminal pressure at the time of visible leakage) observed for non-buttressed and buttressed staple lines. NCEM (137.8 ± 22.3 mmHg), XCEM0005 (109.06 ± 14.14 mmHg), XCEM001 (150.07 ± 15.97 mmHg), XCEM0033 (98.8 ± 10.47 mmHg) and BP (138.8 ± 3.6 mmHg) buttressed staple lines sustained significantly higher mean leak pressures than the non-buttressed staple lines (28.28 ± 10.76 mmHg). However, no statistical differences were observed in the mean leak pressures observed between SIS1 (62.6 ± 11.8 mmHg) and SIS4 (57.6 ± 12.3 mmHg) buttressed staple lines, or when compared to the non-buttressed staple lines. The leak pressures observed with NCEM, XCEM001 and BP were also significantly higher than that observed for both SIS1 and SIS4. The leak pressures observed for NCEM (137.84 ± 22.31 mmHg) and XCEM001 (150.07 ± 15.97 mmHg) were not significantly different from BP (138.76 ± 3.57 mmHg).

All the intestine segments with buttressed staple lines invariably had burst at the mesentery (Figure 3). The burst pressures for porcine intestines varied between 150 and 240 mmHg (Figure 5). There was no significant difference in burst pressures between the different buttress materials and the values were similar to the tissue physiological burst pressure (leak-free control). This demonstrates that there were no statistical differences in the inherent mechanical properties of the intestine segments used between the various experimental groups, indicating that all the buttress materials were able to maintain pressure higher than physiological failure.

DISCUSSION

The overall objective of this study was to evaluate the potential of CEM as a buttress material for staple line reinforcement. The effect of crosslinking of CEM on staple line integrity was also studied. Clinically available small intestinal submucosa (SIS, SURGISIS[®]) and bovine pericardium strips (Peri-Strips[®]) were used as controls. Two configurations of SIS, namely one layer and four-layer SIS were used to study the effect of layering on performance as staple line buttresses. The buttressed staple lines were compared with non-buttressed staple-lines.

Staple line leakage and bleeding are not uncommon problems associated with the use of surgical staplers in gastrointestinal resection and anastomotic surgeries. While there have been advances in stapling device design and surgical techniques, these devastating complications continued to be potential causes of patient morbidity and mortality. Reinforcing the staple lines with buttressing materials has been shown in pre-clinical studies^{17,23,25,26} as well as clinically to be effective in preventing staple line leakage and bleeding.^{4,22,33} In a typical ex vivo setup using porcine small intestine for testing staple line integrity, a continuous pumping of solution exerts increasing intraluminal pressure that leads to the failure of staple lines and/or intestinal tissue.^{17,23} The failure of staple lines starts as a leak. Depending on the rate of leak, either the staple lines fail or the intestines rupture – usually at the mesentery. In this study, the non-buttressed staple-lines invariably failed at staple-lines (Figure 2), while all of the buttressed staple-lines bursted at the mesentery of the intestine (Figure 3).

The results of this study further reiterate that staple line buttressing can improve the staple line integrity and allows intraluminal pressures to reach above physiological

values. CEM, XCEM and BP showed approximately four to five folds higher leak pressures when compared to non-butressed control, while SIS1 and SIS4 buttresses showed approximately two folds increase in leak pressures. When compared with SIS in this ex vivo study, CEM, XCEM001 and BP showed higher pressures before the first observed leak. Although BP is one of the most commonly used buttress material, some concerns regarding long term complications had been raised,³⁴⁻³⁶ as BP strips are effectively non-absorbable and therefore remained for years in the patient. Therefore, there is a need to design buttress materials which can be rapidly absorbed and replaced with site specific remodeled tissue. Our in vivo subcutaneous implantation studies have shown that CEM is rapidly resorbed and replaced by host tissue within 63 days.³⁰

Layering of materials is generally thought to increase the force required to cause mechanical failure.³⁷ Interestingly, in the current study, the use of four-layer SIS did not have any physical advantage over one layer SIS, indicating that a single layer is adequate for reinforcing the staple line. The hypothesized reason for this is that each layer of the layered material perform independently, therefore they do not act as a single unit to improve the overall mechanical strength when used for this purpose. This study also demonstrated that crosslinking of CEM did not have any significant advantage to prevent leakage when compared to non-crosslinked CEM as a buttressing material. However, if a longer in vivo life is desired, CEM can be crosslinked to delay its degradation in vivo.³⁸

Even though this study showed that higher leak pressure was achieved when NCEM and XCEM were used as buttress materials at staple lines, the ex vivo model used was not designed to study their potential in reducing staple line bleeding. Further studies are required to evaluate this functional outcome using more complex in vivo models.

CONCLUSIONS

As a staple line buttress, CEM showed equal mechanical advantage as BP in preventing staple line leakage in an ex vivo porcine small intestine model. Both CEM and BP were shown to be mechanically better than SIS for this purpose. Multi-layered material did not demonstrate advantage over single layer material for buttressing intestinal staple lines. While crosslinking CEM with carbodiimide did not show mechanical advantage, crosslinking may offer functional advantage in providing extended resorption time. This study demonstrated that CEM has potential as a material for staple line reinforcement.

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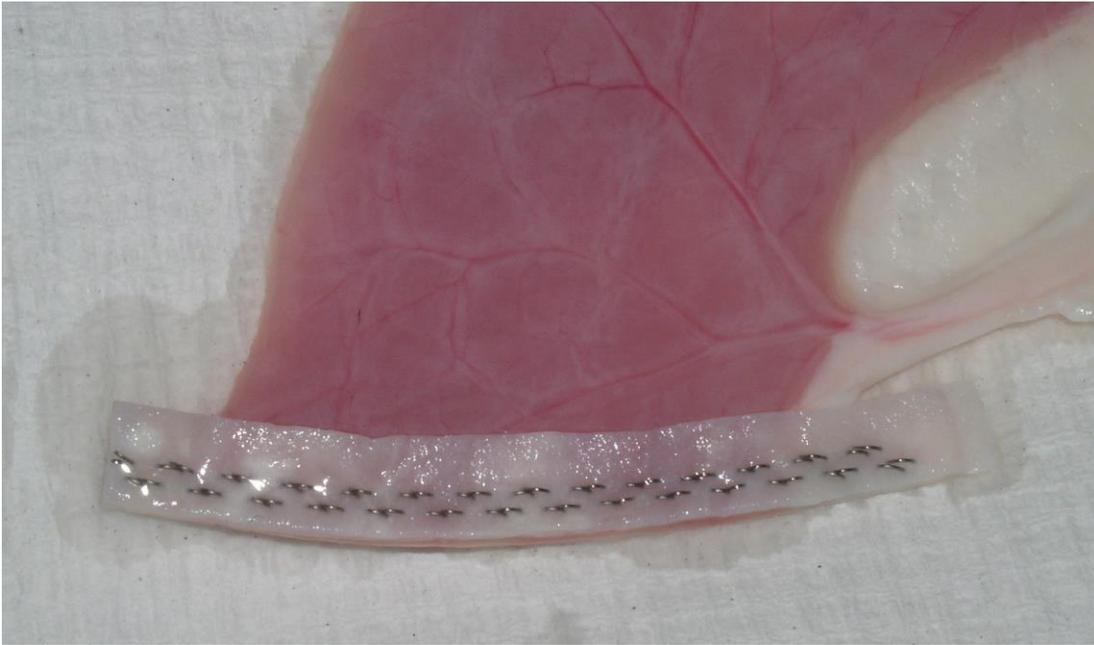
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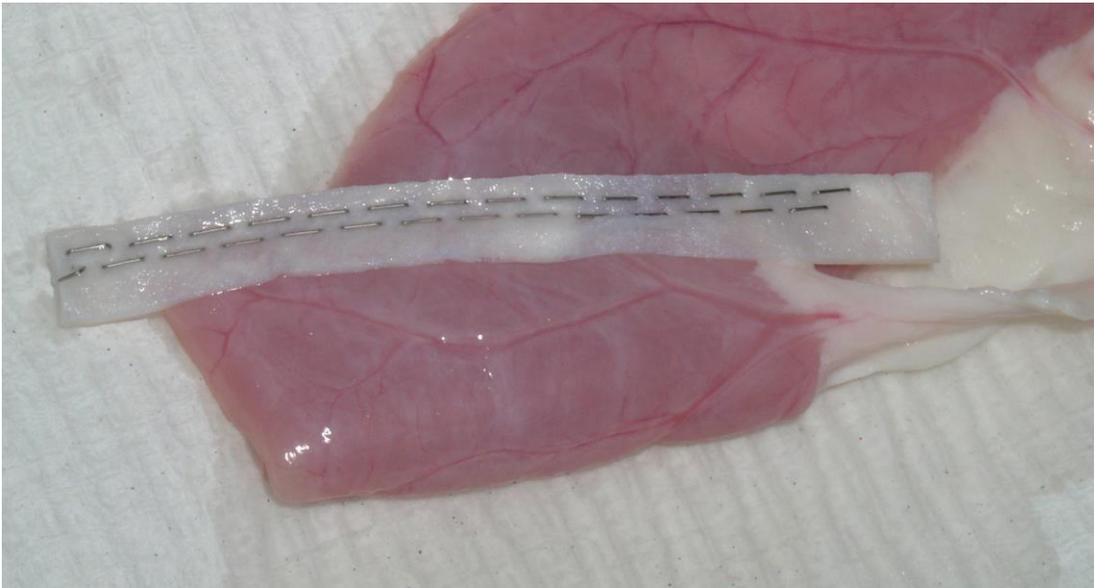
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Table 1: Materials evaluated as staple-line reinforcement buttresses in an *ex vivo* study.

Scaffold Variant	Designation	Source
Cholecyst-derived extracellular matrix (CEM)		
Non-crosslinked	NCEM	Prepared in our laboratory
Crosslinked with 0.0005 mM EDC and NHS/mg CEM	XCEM0005	Prepared in our laboratory
Crosslinked with 0.001 mM EDC and NHS/mg CEM	XCEM001	Prepared in our laboratory
Crosslinked with 0.0033 mM EDC and NHS/mg CEM	XCEM0033	Prepared in our laboratory
Small intestinal submucosa (SIS)		
1 layer	SIS1	Surgisis [®] ; Cook, Inc.
4 layers	SIS4	Surgisis [®] ; Cook, Inc.
Bovine pericardium (BP)		
Glutaraldehyde crosslinked Bovine Pericardium	BP	Peri-Strips [®] ; Synovis Surgical Innovations



A



B

Figure 1A & 1B: Staple line on porcine small intestine segment reinforced with CEM buttress material.



Figure 2: Intestinal segment inflated with Aniline blue dye solution showing leakage at the non-buttressed staple line (→). The leakage rate was too high to allow increase of intraluminal pressure to burst the intestine segment.

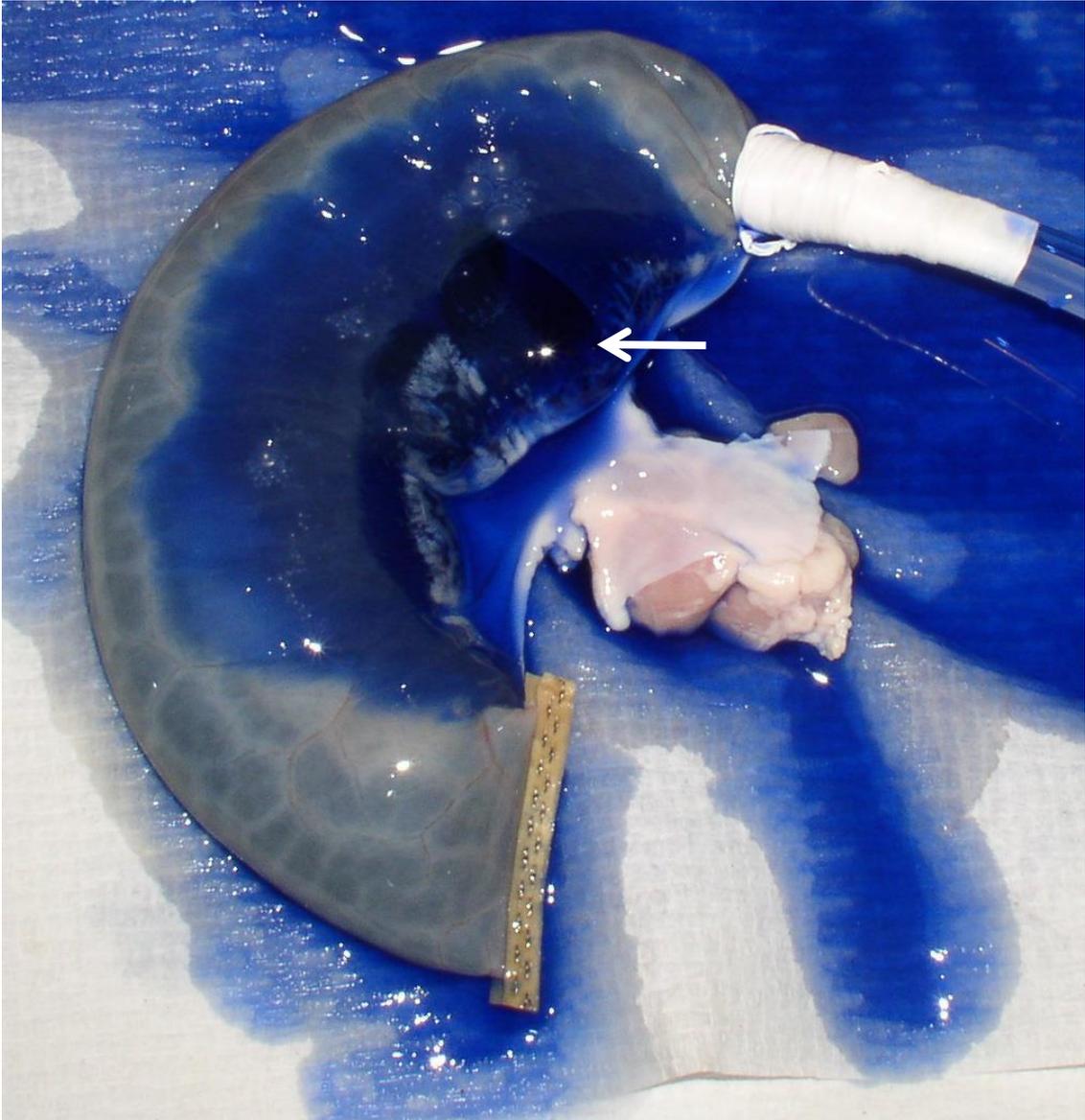


Figure 3: Inflated intestinal segment showing failure at the mesentery (←). The staple line was buttressed with bovine pericardium strip.

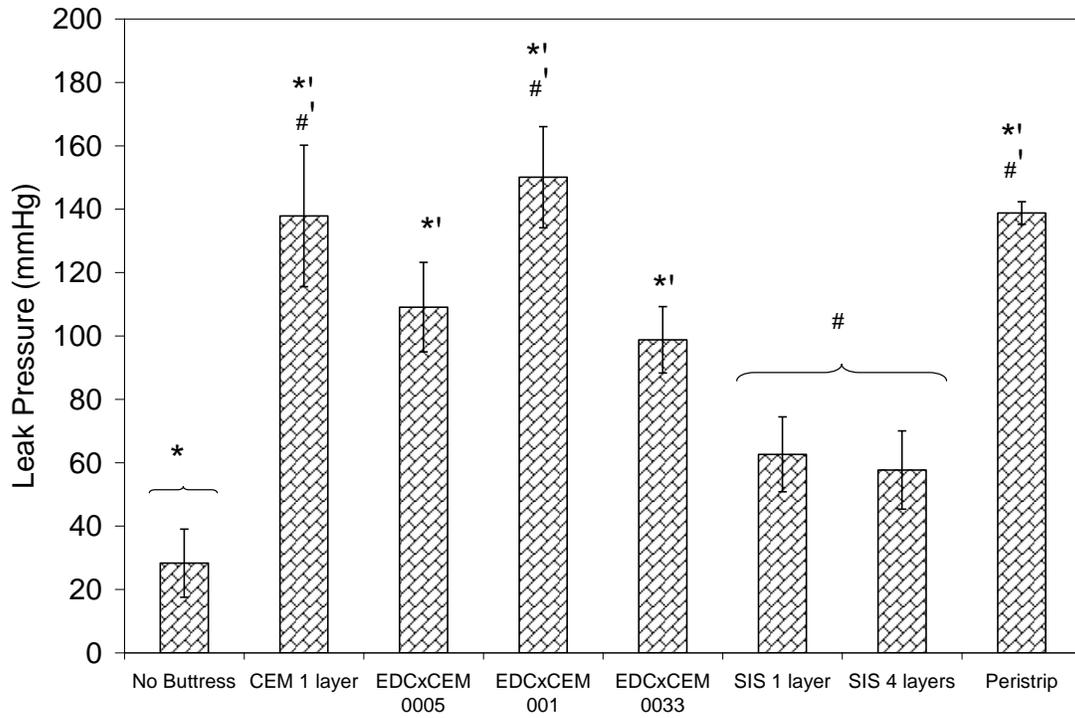


Figure 4: Leak pressures of non-buttressed and buttressed staple lines. * and # indicate statistical differences with *' and #' respectively (p<0.05).

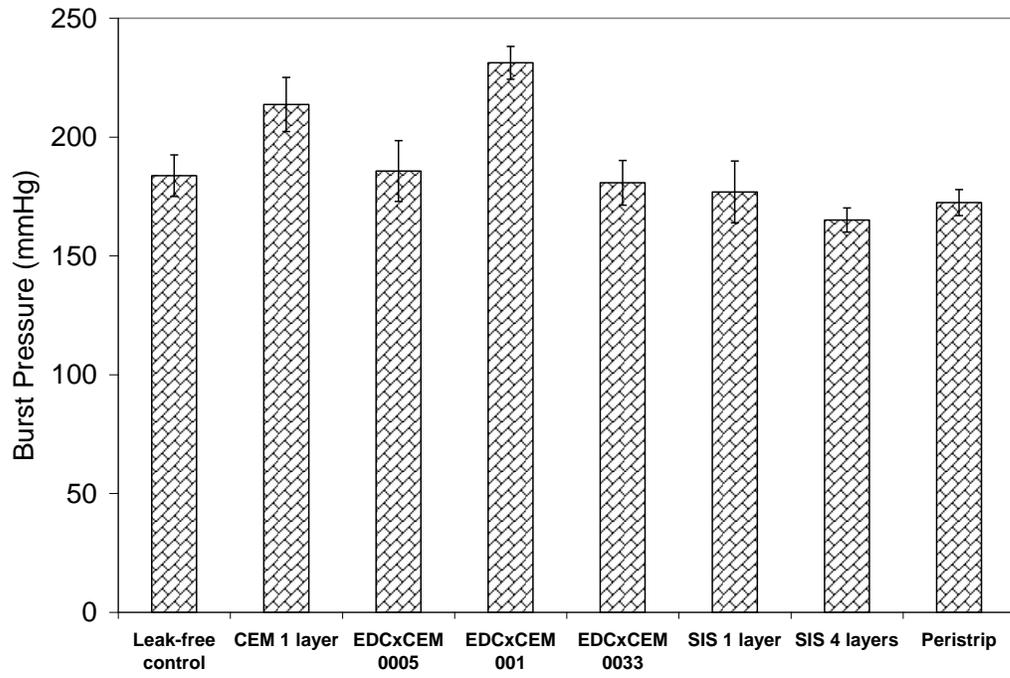


Figure 5: Burst pressures of intestine segments of leak-free control and buttressed staple lines.