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ORIGINAL ARTICLE

A Highly Elastic and Adhesive Gelatin Tissue Sealant for Gastrointestinal Surgery and Colon Anastomosis

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Abstract
Background We describe the development of a highly elastic and adhesive surgical tissue sealant, based on photochemically crosslinked gelatin, for sealing sutured incisions in the gastrointestinal (GI) tract in a rabbit surgical model and in a canine colon anastomosis study.
Methods The study included in vitro assessment of mechanical parameters of the tissue sealant and in vivo analysis of burst strength and histology at 24 h, 3 days and 7 days post surgery, in a rabbit model, to assess progress of wound healing at the suture sites. Utility of this sealant to repair and seal a lower colonic resection and anastomosis procedure in a canine model was also investigated.
Results We show that a photopolymerised gelatin tissue sealant provides effective sealing of GI incisions and facilitates wound healing with no evidence of inflammation up to 28 days post-surgery. Blending of derivatised gelatin with underivatised gelatin allowed tuning of elasticity and elastic modulus of the photopolymerised sealant to suit surgical applications. High tissue adhesive strength was maintained at all blend ratios and exceeded 100 kPa.
Conclusions This highly elastic and adhesive photopolymerised gelatin tissue sealant offers a number of advantages over currently available sealants suitable for GI surgical procedures.

Keywords Tissue sealant · Gelatin · Crosslinking · Photopolymerisation · Gastrointestinal anastomosis · Dehiscence

Electronic supplementary material The online version of this article (doi:10.1007/s11605-011-1771-8) contains supplementary material, which is available to authorized users.

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Introduction

A serious complication following colon anastomosis is anastomotic dehiscence, which may be as high as 13%.[1] Of particular concern is the high incidence of leakage in low colonic anastomoses, which has been reported to be as high as 15–23%,[2,3] with subclinical leak rates reported to be as high as 50%.[4] While estimates of leakage rates in colon anastomoses vary widely, it is associated with high (12.9%) mortality rates.[5] Various methods have been investigated to prevent blood and fluid leakage from the gastrointestinal (GI) tract, including the use of patches, staple line buttresses using both non-absorbable and absorbable

materials and tissue sealants.[6–9] Various tissue sealants have been tested including fibrin glue, with[10] or without a collagen patch,[11] platelet-rich plasma[12] or cyanoacrylate adhesives.[13] A limited number of studies using polyethylene glycol-based adhesives have also been described.[14] Early studies using fibrin sealants to seal colon anastomoses were disappointing, with evidence that the glue inhibited wound healing[15,16] and decreased anastomotic strength, burst strength, and collagen deposition at treated wound sites.[17] Fibrin glues may also inhibit macrophage migration and neutrophil function.[18] Studies with octyl-cyanoacrylates revealed no improvement in healing strength compared to sutured anastomoses, and the cyanoacrylate adhesive appeared to be detrimental at the late phase of healing due to intense inflammatory reactions at the wound site.[13,19] Preliminary studies indicated that aldehyde-crosslinked bovine serum albumin (Bioglue™) adhesive was useful in preventing dehiscence in a colon anastomosis model assayed by burst strength at day 7 post-surgery.[14] Another gelatin-based tissue adhesive that has been used clinically is gelatin/resorcinol/formaldehyde (GRF).[20] Concerns regarding the toxicity and potential carcinogenicity of formaldehyde and glutaraldehyde[21] have led to changes in the formulation of the GRF tissue adhesive and has prevented its approval for use in the USA.

In the light of these previous studies and to address the mechanical and biological specifications required for tissue sealant/adhesives in GI surgery, we report here the superior mechanical and biological performance of a photochemically crosslinked gelatin tissue sealant in both rabbit and canine GI surgical models. The sealant demonstrates high elasticity (>600%) with high adhesive strength (>100 kPa) and excellent tissue integration and healing response. Combined with the low cost of components, this new material displays many requirements of an "ideal" GI surgical tissue sealant namely safety and efficacy, ease of use and effective sealing, low cost, and compliance with regulatory requirements.[22]

Materials and Methods

Materials

Gelatin, porcine type A, ~300 g Bloom (G1890); chemicals, including the metal complex tris(2,2′-bipyridyl) dichlororuthenium(II) chloride hexahydrate $(RuII(bpy)_3)^{2+}$, sodium persulphate and Bolton-Hunter Reagent (BH reagent; *N*-succinimidyl-3-4-hydroxyphenyl propionate) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Collagen membrane (dried sausage casings) were from The Brew Shop, Adelaide, South Australia. All other chemicals were of analytical grade and were purchased from Sigma-Aldrich unless otherwise stated. Phosphate-buffered saline (PBS) was from Invitrogen Australia, Melbourne, Australia.

Gelatin Derivatisation and Formulation

Gelatin was derivatised to increase its tyrosine (phenolic) content, using BH reagent as previously described[23,24,26] Blends of derivatised and underivatised native porcine gelatin were formulated by combination in the solid state at designated blend ratios, then formulated to 17.5% concentration in PBS (pH 7.4) containing 0.1% Tween 80.

Photochemical Crosslinking

A photochemical process, described previously[25,26] was used to crosslink gelatin for swelling analysis, mechanical testing, and for surgical applications to repair tissue defects.

Swelling Analysis

Swelling analysis of photopolymerised cast discs (13 mm diameter×2 mm) was carried out as described previously.[26]

Mechanical Analysis

Adhesive and tensile properties of cast gelatin mixtures were measured using an Instron 5544 mechanical tester as previously described.[26] Each formulation was tested in quadruplicate and mean and standard deviations were calculated.

Burst Strength Analysis in Nonhealing Models

Two in vitro nonhealing models were developed to determine photopolymerised gelatin sealant performance. The first was designed to simulate repair of tissue where there is low strain (for example, dura or vascular sealing) and the second was designed to measure performance under moderate strain conditions (~ 300%) to simulate repair of elastic tissues such as gastrointestinal (GI) and lung.

In the first low-strain model (e.g., dura/vascular) a piece of collagen membrane was fixed over the open end of a 19-mm diameter polypropylene cylinder to create an airtight seal using rubber bands. Using a 12-G cannula needle, three holes (each approximately 3 mm in diameter) were created in the membrane. The entire surface of the 19 mm collagen membrane was then covered with a single application of 200 μl of gelatin sealant, followed by photopolymerisation, to yield an application thickness of approximately 1 mm. The sealed membranes were then fully immersed in a PBS (including 0.04% sodium azide) bath at 37°C for up to 7 days. Up to six membranes were used for burst test analysis at 1 min (T0) and then at 1, 3, 5, and 7 days after immersion. The test system, filled with PBS, with the affixed collagen membrane was gradually pressurised (at ~2 mm Hg/s) until the membrane or applied sealant failed. The burst strength was

recorded using a digital pressure sensor (digital manometer; Sper Scientific, John Morris Scientific; Sydney, Australia).

The high strain (e.g., GI and lung) in vitro model consisted of collagen tubing (30 cm length,15 mm diameter) hydrated for 2 h in PBS into which a single 5-mm longitudinal defect was made using a scalpel blade. The defect was sealed by application of 250 μl of gelatin formulation followed by photochemical crosslinking. No suture was used in these tests and burst strength performance was dependent solely on the mechanical strength of the sealant. The sealed collagen tubes were filled with PBS, the ends tied off and incubated submerged in a sealed PBS (including 0.04% sodium azide) bath at 37°C over a period of 7 days during which four samples were removed at various time points (0, 1, 3, 5, and 7 days) for burst strength testing (described above).

Rabbit Surgery—Ileum Defect Repair

Healthy New Zealand white rabbits were used for in vivo analysis of tissue sealant performance in a colon anastomosis model. This study was approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Animal Care and Ethics Committee (approval number 09/12). Animals were acclimatised as a group for 7 days in free-range pens. All rabbits received 1 mL enrofloxacin (Baytril, 12.5 mg/kg IM 26 G needle) for 5–7 days prior to surgery. Prior to surgery, animals were premedicated with buprenorphine (0.03 mg/kg IM 26 G needle) and midazolam (1.3 mg/0.25 mL IM 26 G needle) 15 min before anaesthetic induction. Rabbits were induced with 5% (*v*/ *v*) isoflurane followed by 2–4% isoflurane maintenance with 3 L/min delivered via a tight-fitting face mask. The ventral side of the rabbit was shaved and the skin disinfected with surgical iodine. A 5–7 cm incision was made on the ventral midline, the ileum was isolated and placed on to a moist sterile dressing. Up to 12 defects, a total of six defects per treatment, 10 cm apart, were created by inserting a no. 12 scalpel blade into the ileum to a predetermined depth, creating a 5-mm longitudinal defect. A 6/0 suture (Safil®, B. Braun, polyglycolic acid, braided, coated, absorbable) tied with one flat knot was used to close the centre of the defect.

The gelatin sealant solution (250 μl) was applied over the sutured defect site and was photopolymerised for 30 s using a xenon light source. The ileum was placed back into the abdominal cavity and the muscle layer closed with 2/0 (Dexon™ 11, USS-DG) absorbable sutures (single interrupted) and skin layer closed using 3/0 sutures (Vicryl™, Ethicon; single interrupted). Dextrose saline (4% dextrose, 0.18% saline) 15 mL/kg subcutaneous injection was given to aid recovery. Rabbits were given post-operative analgesia (buprenorphine 0.03 mg/kg IM 26 G needle) as required. Rabbits received 1 mL enrofloxacin (12.5 mg/kg IM 26 G needle) for a further 7 days after surgery.

At various time points post surgery (from 0 to 7 days), three animals from each of the six groups (six defects for each formulation, two formulations per animal) were anaesthetised and the ileum gently removed and assessed. The animals were then euthanized using Lethabarb™ (sodium pentobarbitone 325 mg/mL) 0.5 mL/kg directly into the heart. The ileum was excised and the condition of the photopolymerised gelatin sealant was assessed visually. Burst strength analysis, described earlier, was conducted on four independent sealed defects for each sealant formulation treatment and tissue samples from an additional two independent sites were taken and fixed in formalin for subsequent histological analysis.

Burst Strength Analysis and Surgical Biopsies

The burst pressure of repaired defects was measured using a custom-made device. Segments of ileum approximately 75 mm in length containing the sealed defect were carefully removed and ligated at each end with coarse nylon thread. The lumen pressure (measured as mm Hg) was increased to burst point at a rate of 1 mm Hg per second using a sphygmomanometer bulb attached to a digital pressure sensor (digital manometer; Sper Scientific, John Morris Scientific, Sydney, Australia) with air introduced via an 18 G IV catheter. Failure of the repaired wound or tissue was detected by air bubble leakage from the tissue submerged in a bath of PBS and the maximum luminal pressure was recorded as the repaired wound burst strength.

Canine Colon Anastomosis Repair

The efficacy of crosslinked gelatin as a tissue adhesive was also tested in a canine colon anastomosis surgical model. The procedure performed was a trans-anal stapled anastomosis of the distal colon. Four dogs (4-year-old greyhounds) were used in these experiments, which were carried out according to CSIRO and University of Melbourne Animals Ethics protocols. A standard bowel prep consisting of fasting, laxatives (Golytely), and enemas was performed prior to surgery to empty and clean the distal colon.

The dogs were premedicated with buprenorphine (0.01 mg/kg s.c.), then anaesthetised with thiopentone (20 mg/kg i.v.) via the jugular vein. The dogs were intubated and anaesthesia maintained with 1.5–2% isofluorane and mechanically ventilated at 1.5 L, 250 mm H_20, 8 bpm, and 800–1,000 mL tidal volume. The abdomen was clipped and the skin prepared with serial applications of an antiseptic and detergent solution (Savlon™—chlorhexidine and cetrimide),

70% alcohol and finally the antiseptic chlorhexidine. Prophylactic antibiotics (cephalothin 12 mg/kg) and anti-inflammatory (carprofen 2 mg/kg) drugs were administered prior to the procedure.

A ventral midline abdominal incision approximately 200–250 mm in length was made to exteriorize the descending colon and a segment of the descending colon isolated with laparotomy pads. At the site of the planned anastomosis, the vasa recta of the left colic artery and vein contained within a 20–30 mm segment at the planned site of the anastomosis were ligated with suture and transected. The anastomotic site was located approximately 100–120 mm from the pelvic brim, approximately midlength within the descending colon. Intestinal sizers were inserted trans-anally to measure the diameter of the intestinal lumen to determine the appropriate stapler size to be used. A purse string suture was placed on either side of the proposed anastomosis using a purse string device and Prolene (size 2–0) on a KS needle. The appropriately sized Endopath ILS Endoscopic Curved Intraluminal Stapler (21 mm; Ethicon Endo-Surgery, Inc) was coated with a water-soluble lubricant and inserted trans-anally and delivered to the planned site of anastomosis. A 25-mm section of colon was transected between the purse string sutures and the two ends rejoined using the intraluminal stapler as per instrument instructions.

After firing of the staples, the firing handle was released and the assistant re-engaged the safety. The instrument was opened by turning the adjusting knob one half to three quarters of a revolution. The instrument was rotated 90° in both directions to ensure the anvil was free from tissue. The stapler was then withdrawn by gentle pulling and rotation, and the anvil then removed from the stapler. The washer (if present) and tissue donuts from within the circular knife were removed and the integrity of the tissue donuts examined.

Once the anastomosis was completed, a bubble leak test was performed by positioning intestinal forceps approximately 40–60 mm proximal and distal to the anastomosis and insufflation with air via a 22 G catheter until the walls of the colon were moderately distended and any large leaks sealed by using an interrupted suture pattern with 4–0 Vicryl sutures (Ethicon; NJ, USA). Approximately 1 mL of gelatin sealant was applied to the circumference of the rejoined colon junction to cover and seal any remaining leaks. This was performed in two applications, 500 μl each for both superior and inferior surfaces and photopolymerised for 45 s using a xenon endoscope lamp. The sealed defect was examined visually before closing the abdomen to ensure there was no leakage. The animals were allowed to recover and were euthanised after 2 or 4 weeks to assess the anastomosis site. The tissue surrounding the incision site was evaluated macroscopically, then excised and processed for histology.

Histology

Histological analysis was performed on the surgical defect sites of the ileum of each rabbit and colon of each dog used in the surgical analysis of the gelatin sealant. In the rabbit ileum study, duplicate surgical defects and repair sites were made in addition to the sites for burst strength analysis for the specific use of histological analysis. In the dog colon, only a single anastomosis site was performed and used specifically for macroscopic and histological analysis. Photographs were taken of the luminal and serosal surfaces and tissue samples taken for histology. Samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Cross-sections (5–10 μm thick) were mounted on glass slides and paraffin was removed by xylene. Sections were then rehydrated through decreasing concentrations of ethanol and stained with hematoxylin and eosin (H&E).

Statistical Analysis

Any differences reported as significant were determined by analysis using Student's *t* test with two-tailed distribution tails and two-sample equal variance. *P* values less than 0.05 were considered significant.

Results

Tissue Sealant Mechanical Properties

Swelling

Swelling of the photopolymerised gelatin sealant was tunable via phenolic derivatisation (using BH reagent) of endogenous lysine residues. Unmodified gelatin swelled to 100% of its initial volume within 24 h and became weak and eventually dissolved over 12 days (288 h) when incubated at 37°C in PBS (data not shown). This swelling was effectively controlled by blending unmodified gelatin with BH-derivatised (phenolic-derivatised) gelatin. Phenolic derivatisation of the gelatin thus facilitated controlled tuning to produce a material with a defined swelling endpoint.

Tensile Properties

The elasticity of the photopolymerised gelatin sealant varied depending on the proportion of BH-modified gelatin. One hundred percent BH-modified gelatin had an extension to break (E_b) of 198% compared to 672% for 100% unmodified gelatin and the E_b of the gelatin sealant decreased in response to increasing the content of BH-modified gelatin (data not shown). Blending the proportion of phenolic-

derivatised gelatin to unmodified gelatin thus allowed fine-tuning of sealant extensibility. We have shown previously that the elastic modulus of the fully derivatised gelatin was about fivefold higher than the underivatised gelatin.[26]

Adhesive Properties

There was no significant difference in the adhesive strength of the gelatin blends compared to unmodified gelatin with all blends having adhesion strengths of approximately 100 kPa (data not shown).

In Vitro Low-Strain Dura/Vascular Model

Burst Strength Analysis Burst strength of 165 mm Hg (±5.1) was maintained after immersion in PBS at 37°C over a 7-day period using a formulation comprising fully derivatised gelatin (Fig. 1a). The unmodified gelatin showed the most reduction in burst strength within the first day, and this reduced to ~77.6 mm Hg (±10.1) over 7 days. There were no significant differences between the burst strength of each blend, with blends of 1:1, 1:3, and 1:4 demonstrating the highest burst strength over the 7-day period. The failure at burst was due to cohesive failure of the sealant with no delamination of the sealant from the membrane noted.

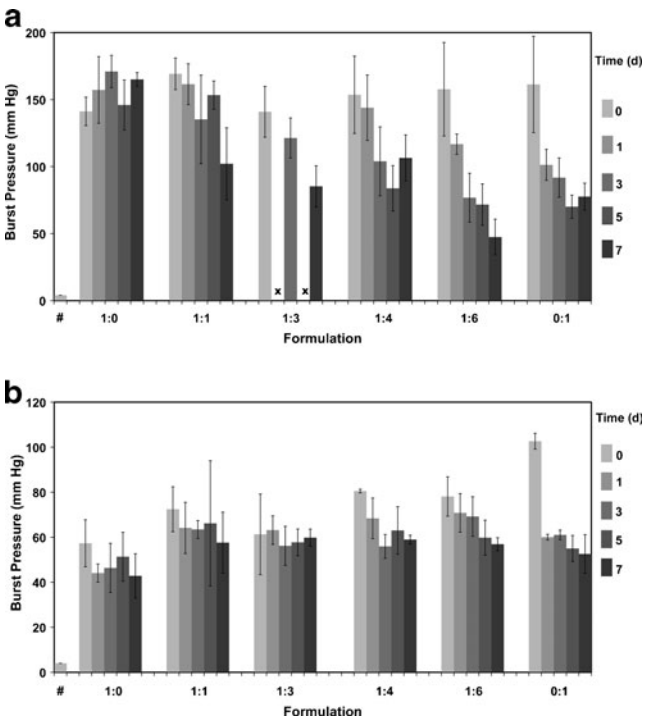

Fig. 1 Burst strength pressures of the gelatin sealant formulations over a 7 day period in (**a**) An in vitro "low-strain Dura" collagen membrane model, and (**b**) An in vitro "high strain GI" collagen tube model. "X" denotes no datapoint due to dissolution of the sample. Ratios indicate blends of modified (BH-derivatised):unmodified gelatin

In Vitro High-Strain GI/Lung Model

Burst strength analysis The gelatin sealant maintained mechanical strength over a 7-day period in a nonhealing in vitro GI model that measured sealant performance under moderate tissue elasticity (~300% strain; Fig. 1b). Only marginal loss of burst strength, maintained at ca. 60 mm Hg, was recorded for most formulations tested over a 7-day period with immersion in buffer solution at 37°C. No significant differences were observed between the burst strength of any particular formulation (Fig. 1b), which indicated that it was possible to control the degree of swelling independent of mechanical integrity. Failure at burst was due to cohesive failure of the sealant. These data demonstrated that the mechanical properties of the photo-polymerised sealant were responsible for the effective tissue sealing (to greater than ca. 60 mm Hg pressure) over at least a 7-day period. During this time, natural healing processes would occur in vivo.

Rabbit GI Surgery

Burst Strength Analysis

In order to assess both the mechanical strength of the gelatin sealant and the effect of sealant application on wound healing in vivo, a rabbit ileum defect repair study was undertaken. Burst strength analysis was carried out over a 7-day period including T0 (immediately after sealant application), then at 1, 3, and 7 days after sealant application. At each time point, the animals were euthanized and the ileum removed for burst strength and histological analysis. Figure 2a shows the ileum retrieved 7 days after surgical repair of 12 independent defects using various formulations of the gelatin sealant. All 12 sites showed intact and strongly adhered sealant. Burst strength of both native ileum tissue and of defects repaired with sutures only were measured to determine both the rate of the healing process and mechanical strength of the tissue. These two measurements were used as baseline controls. At various time points, up to 7 days, sealant-repaired defect sites were tested for burst strength.

Immediately after application of the sealant ($T=0$), all sealant blends showed burst strengths significantly ($p<0.05$) higher than defects repaired with sutures alone (Fig. 2b), with the 1:3 blend of BH-derivatised gelatin:unmodified gelatin showing the highest burst strength. Failure at burst was observed as cohesive failure of the sealant with no cases of delamination noted. The 1:3 blend showed burst strengths in the order of 75 mm Hg (±22.8 mm) compared to 21 mm Hg (±7.5) for suture only repairs. After 24 h, the burst

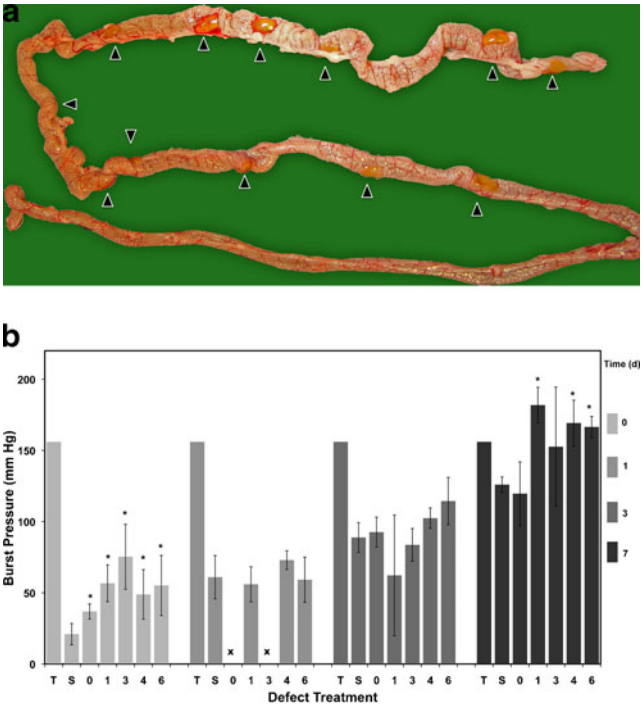

Fig. 2 Rabbit ileum defect repair using the gelatin sealant formulations over a 7 day period. (**a**) Ex vivo rabbit ileum removed 7 days post surgical application of various formulations of the gelatin sealant applied to seal and repair 12 surgical defects. Arrows indicate presence of sealant at each defect site, and (**b**) Burst pressure of rabbit ileum sealed with different formulations of the gelatin sealant; T = native undamaged tissue; S = sutured defect with no sealant; 0= 100% BH-derivatised gelatin; 1=1: 1 blend of 1 part BH-derivatised gelatin with 1 part unmodified gelatin; 3=1:3 blend of 1 part BH-derivatised gelatin:3 parts unmodified gelatin; 4=1:4 blend of 1 part BH-derivatised gelatin:4 parts unmodified gelatin; 6=1:6 blend of 1 part BH-derivatised gelatin:6 parts unmodified gelatin. Asterisks show significant ($p<0.05$) differences in burst strength from defects repaired using sutures only (S)

strength of defects repaired with sutures only had increased to 61 mm Hg (±15 mm) indicating that natural healing and fibrin formation had commenced. There was no significant difference ($p<0.05$) in burst strengths obtained from sealant-treated defects at this time point. At 3 days post-surgery, the healing process proceeded normally, based on increasing burst strengths, and was essentially complete by 7 days, with all defects showing burst strength pressure equivalent to, or exceeding, native ileum tissue. The 1:1, 1:4, and 1:6 blends showed burst strengths significantly ($p<0.05$) higher than the suture-only treatment 7 days post-surgery. These results show that application of the cured tissue sealant over the defects had not compromised the normal wound healing response and that by day 7, complete healing of the defects had occurred. Rapid healing of defect sites, with application of all sealant formulations, prevented specific assessment of the mechanical performance of the sealant alone.

Histological Analysis

Figure 3 shows both gross morphological and histological analysis of sealant-treated ileum wound sites, excised at time points described above ($T=0$, 1, 3, and 7 days post-surgery). The top panels show the (a) serosal and (b) luminal surfaces of defect sites removed from animals at each time point ($T=0$, 1, 3, and 7 days post-surgery). There was no apparent inflammation noted, nor evidence of post-surgical bleeding or leakage of luminal contents. The middle panels (C) show histological cross-sections with the cured (1) sealant layer, (2) applied over the ileum at (3) the defect site. A serosal epithelium had grown over the cured sealant layer, which was seen as early as day 1 post-surgery (panel D). There was no evidence of macrophages or lymphocytes at the wound site, nor evidence of foreign body giant cell formation. There was also close apposition of the cured sealant layer to the serosal surface of the ileum that was maintained over the time course of this study (panel E) noted in all sealant-treated defect sites ($n=30$). By day 3, there was evidence of cellular infiltration into the cured sealant layer and evidence of small lacunae with resultant local degradation of the sealant.

Canine Colon Anastomosis

Histological analysis of the effect of photopolymerised gelatin sealant application to the canine colon anastomosis site at 2 weeks post-surgery revealed a chronic fibrosis extending through tunica muscularis, submucosa, and tunica muscularis mucosae. The external morphology and gross pathology was uneventful, with good healing apparent at the surgical repair site (Fig. 4a). There was no evidence of post surgical dehiscence or subsequent inflammation. Severe chronic fibrosis was restricted to the surgical site (Fig. 4b). Dense scar tissue extended from the subserosa through the tunica muscularis (where it was associated with severe atrophy of smooth myocytes of both the longitudinal and circular coats) into the submucosa. Focally, scar tissue obliterates the tunica muscularis mucosae and extends into the deep lamina propria of the mucosa. There was moderate dilation of the deep gland crypts in the latter area with focal herniation of a dilated crypt into the underlying submucosa (Fig. 4c). Only sparse small lymphocytes and occasional erythrophagocytosing macrophages were scattered in the scar tissue. There was no significant inflammation apparent.

Discussion

Increasing the phenolic content of gelatin, via conversion of endogenous lysine residues to tyrosine-like residues using

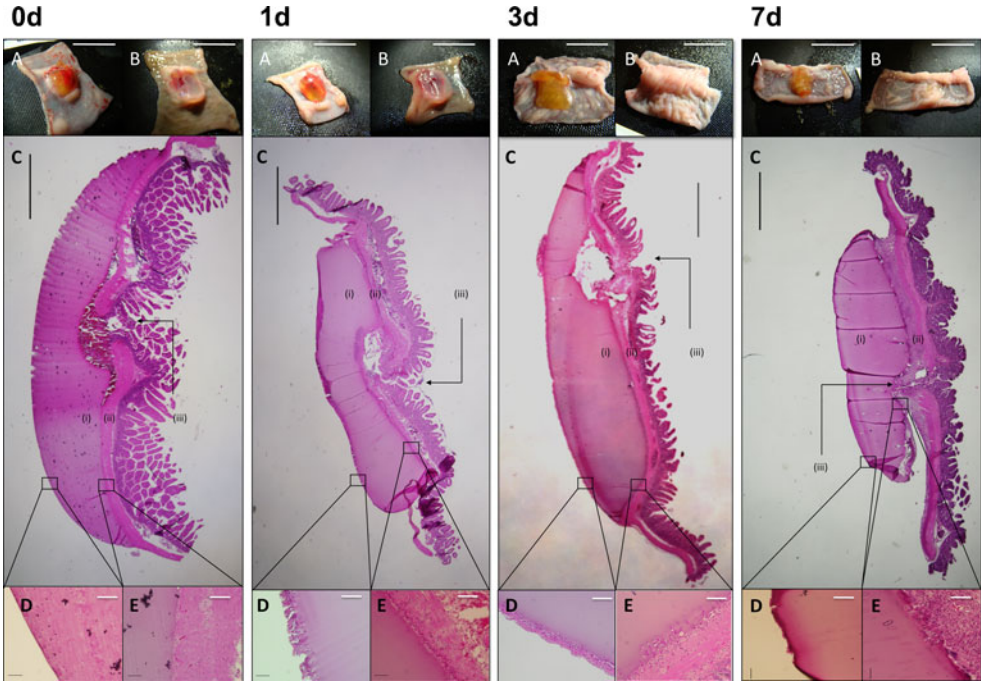

Fig. 3 Rabbit ileum with 3–5 mm defect sealed with the gelatin sealant application. Images show histology of sealed defects, at 0 h, 1-, 3- and 7-days post surgery (H&E stain). (**a**) Serosal surface; (**b**) luminal surface; (**c**) transverse section of defect site, (i) photopolymerised sealant layer, (ii) ileum, (iii) defect site; (**d**) outer surface of photopolymerised gelatin sealant layer; (**e**) ileum serosal surface with photopolymerised gelatin sealant attached

Bolton-Hunter reagent, reduces post-swelling behaviour of the photocrosslinked gelatin hydrogel, due to increased dityrosine formation. Uncrosslinked gelatin swells considerably, eventually dissolving due to increasing hydrostatic load.[27] These studies confirm the prediction that regulation of hydrogel swelling, via interchain crosslinking, is an important criterion for the maintenance of functional sealant performance in vivo.[28] Swelling affects elasticity, cohesive tensile strength, and tissue adhesive strength and recent publications noted serious post-surgical problems (nerve compression), resulting from excessive swelling of a crosslinked synthetic hydrogel used to repair dural defects.[29,30] DuraSeal™ was shown to swell up to 98% within 24 h, while CoSeal™ swelled to over 500% in 3 days in PBS.[31]

The mechanical properties of the photochemically crosslinked gelatin sealant and blends containing derivatised gelatin were measured using a variety of tensile and adhesive tests. The adhesion strength to collagen membrane did not vary significantly at any blend ratio (from unblended gelatin to fully phenolic-derivatised gelatin). Similarly, there was a slight effect of the extent of phenolic derivatisation on the tensile strength of the photo-crosslinked material, with fully derivatised gelatin showing lower ultimate tensile strength. The strain to break, however, was significantly affected by the ratio of phenolic derivatisation. Increased phenolic content resulted in a stiffer crosslinked material, with a higher elastic modulus, due to increased crosslink density and the extension to break was reduced from >600% to <200%. We have shown previously that the adhesive strength of the photochemically crosslinked sealant was greater than both other gelatin- and fibrin-based tissue sealants.[26] In the present study, the burst strength of the photochemically crosslinked gelatin sealant described here also exceeds the burst strength reported for GI defects sealed with BioGlue®[32] a tissue sealant that had been trialed previously in colon anastomosis procedures.

Bio-Glue® is a biological protein-based material consisting of 45% bovine serum albumin solution and 10% glutaraldehyde that was approved by the US Food and Drug Administration in 1999 for use in the repair of acute aortic dissections. While displaying good adhesion properties, crosslinked BioGlue® is a brittle material displaying at most 13% strain to break[33,34]. For use in elastic tissues, such as lung and GI tract, it is not well matched to the mechanical properties of the tissue to which it is applied.[35] In addition, there are also toxicity and safety issues associated with use of this product.[36] For example, it has been associated with acute

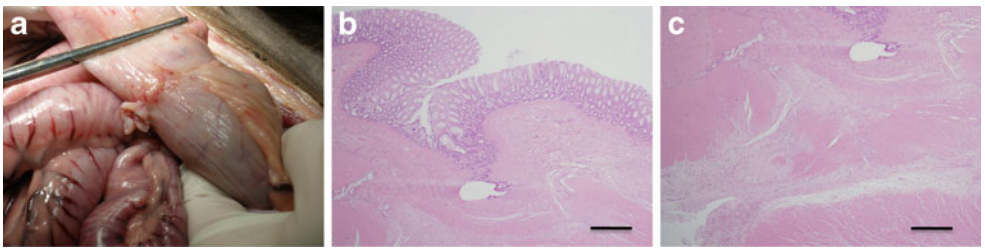

Fig. 4 Canine colon anastomosis 2 weeks post surgery. (**a**) External morphology of the surgical site; (**b**) and (**c**) H&E stained transverse sections through the anastomosis site. (*Bar* 100μm)

intraoperative malfunction of aortic valves,[37,38] acute limb ischemia,[39] and fatal myocardial infarction due to embolization.[40] A recent report also described a pulmonary embolism associated with the use of BioGlue® in repair of an aortic dissection.[41] In addition, a recent report described a foreign body reaction to BioGlue® in which a chronic granulomatous inflammatory response to the tissue adhesive was identified as the cause of the pericardial effusion and cardiac tamponade.[42]

Similarly, toxicity and tissue inflammation have been observed with the GRF tissue adhesive. For example, Bonchek et al.[43] showed a band of necrosis and inflammation adjacent to the zone of GRF adhesive in a dog kidney surgical repair. In a large study[44] of several tissue adhesives in porcine infrarenal aorta dissection, it was demonstrated that GRF glue caused the most severe histopathological disruption of the aortic wall structure, exceeding both BioGlue® or the fibrin-based sealant Tissucol™ in this respect. These effects included stenosis, elastinolysis, a decrease in elastic elements of the wall, an increase in infiltration of T lymphocytes and macrophages, and aberrant distribution and density of the network of small blood vessels (*vasa vasorum*).

Preliminary studies using a microbial transglutaminase-crosslinked gelatin sealant have also been described.[45] While the cured sealant displayed good cohesive/adhesive and burst strength in vitro, its curing time was relatively slow (up to 5 min) and the safety and biocompatibility of microbial transglutaminase in the final formulation has not been extensively studied.

The in vivo tissue compatibility of the present gelatin sealant is demonstrated by the rapid growth of serosal epithelium over the applied photopolymerised sealant. As shown in Fig. 3, by 24-h post surgery, a multicellular layer of serosal cells is apparent on the external surface of the sealant layer. The close apposition of the photopolymerised sealant and the rabbit ileum tissue is also apparent from initial application to 7 days post-surgery with no evidence of sealant dehiscence. These histological results support the mechanical performance of the sealant (Fig. 2) that would result from strong covalent crosslinking of the photopolymerised sealant to the tissue. Rapid wound healing of the ileum defects in vivo prevented the specific assessment of the mechanical performance of the sealant. However, in a nonhealing in vitro model, the photopolymerised sealant remained firmly attached to submerged collagen membrane for 7 days (at 37°C) and was able to withstand internal burst strength exceeding 60 mm Hg over that duration. No delamination of the sealant was observed. Furthermore, the elasticity of the photopolymerised gelatin sealant more than adequately accounts for the known elasticity (extension to break) of colon tissue, reported at >220% in rat colon.[46] While it is not possible to assign wound-healing enhancement to the sealant application, the results presented in this study show that there is no inhibition of wound healing in the presence of photopolymerised gelatin sealant.

Application of the photopolymerised gelatin sealant to the serosal surface of canine colon did not cause an observable inflammatory or foreign body (giant cell) response up to 7 days post-surgery (Fig. 4). Furthermore, there were no other complicating histopathological events (intense, focal acute, or chronic nongranulomatous inflammation) seen in the gelatin sealant-treated colon tissue in the course of this study. Lack of inflammation in the peritoneum also indicated patency of the colon anastomosis provided by application of gelatin sealant. The attached movie (Movie 1) shows a canine colon anastomosis procedure carried out using an ILS stapler and sutures. Following photopolymerisation, the gelatin sealant was shown effectively to seal the surgical site preventing leakage of intraluminal liquid from residual small defects at the stapled and sutured anastomosis site.

The gelatin-based photochemical tissue sealant described here displays high adhesive and burst strength together with high elasticity, properties which are attained within seconds of illumination with white light, using nontoxic, biocompatible and biodegradable components. These physical specifications, combined with favourable biocompatibility, offer significant advantages over tissue sealants currently available for GI surgical procedures.

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