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Comparison of covalently and physically cross-linked polyethylene glycol-based hydrogels for the prevention of postoperative adhesions in a rat model

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A covalently and a physicochemically cross-linked hydrogel, both based primarily on polyethylene glycol and both formed *in situ*, were compared side by side in a rat uterine horn devascularization and serosal injury model for efficacy in adhesion prevention. The primary difference between the two materials was the nature of their cross-linking. The covalently cross-linked hydrogel was a photopolymerized polyethylene glycol-*co*-lactic acid diacrylate, and the physically cross-linked hydrogel was a polyethylene glycol-*co*-polypropylene glycol, Poloxamer 407®. In the surgical model employed, application of the covalently cross-linked hydrogel reduced the extent of adhesion formation from 75 ± 10% in the control group to 16 ± 6% (mean ± s.d., $P < 0.001$). Application of the physically cross-linked hydrogel reduced adhesion formation to 38 ± 19% ($P < 0.01$). Retention of the two hydrogels upon the site of application was also evaluated. The covalently cross-linked hydrogel formed a continuous barrier upon the uterine horns for more than 4 d, while the physicochemically cross-linked hydrogel was present upon the uterine horns for less than 2 d. This difference in retention was probably the cause of the difference in efficacy and may be attributed to the nature of the cross-linking.

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Postoperative adhesion formation is a frequent surgical complication that can cause pain, bowel obstruction and infertility. Postoperative adhesion formation has been problematic in many surgical specialities, including gynaecological, orthopaedic and cardiovascular surgery. In abdominal and pelvic surgery, damage to the mesothelium and stroma during surgical manipulation causes the release of histamine and vasoactive kinins. These substances may cause an increase in capillary permeability, leading to the formation of a serofibrinous exudate[1]. In normal tissues, plasminogen activators secreted by the mesothelium activate plasmin to quickly lyse this exudate. Damaged, ischaemic peritoneal tissues, however, display decreased levels of plasminogen activator secretion[2], and thus the exudate is not lysed, permitting a fibrin matrix to form bridges between adjacent tissues and organs. Over time, these matrices become ingrown with collagen-secreting fibroblasts, and the adhesions become vascularized, resulting in permanent scar tissue bridges which may restrict normal organ movement and obstruct blood supply[1].

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A number of modalities for adhesion prevention have been investigated. These have fallen primarily into two categories: pharmacological agents and barrier materials. Pharmacological agents used have included anti-inflammatories[3], antihistamines[4] and fibrinolytic agents[5]. Barrier materials have been used to mechanically separate tissues from one another. They may also prevent the shedding of plasminogen activators from the near surface of the mesothelium, resulting in a localized increase in the concentration of plasminogen activators at the tissue surface[6]. Some of the materials that have been investigated for use as barrier materials have included oxidized regenerated cellulose[7], expanded polytetrafluoroethylene[8] and hyaluronic acid[9]. Ideal barrier materials should have good biocompatibility, be easy to apply, be conformal and adherent to the underlying tissue, and degrade within a reasonable period of time so that surgical removal will not be necessary.

Our laboratory has recently reported the use of a hydrogel barrier material that is applied to tissues as a liquid and converted to a hydrogel *in situ* by exposure to long-wavelength ultraviolet light for the prevention of postoperative adhesions[10]. This hydrogel adhesion

barrier has previously been evaluated in rat uterine horn devascularization and adhesiolysis models[10] and in a rabbit overian wedge resection model[11]. In all three models examined, the hydrogel barrier provided a significant reduction in adhesion formation. In the rat uterine horn devascularization and serosal injury model, the hydrogel barrier reduced adhesion formation by 87%[10].

This hydrogel is formed from a water-soluble, macromolecular precursor which consists of a polyethylene glycol central chain (MW 8000) copolymerized at each end with DL-lactide, having an average of five hydrolysable lactyl residues per end, and further capped at each end with an acrylate group[12]. The hydrogel is formed by coating tissues with an aqueous solution of the precursor that contains a photoinitiator and then briefly exposing the coated tissue to long-wavelength ultraviolet light. The resultant hydrogel barrier is conformal and adherent to the tissues upon which it was formed, presumably due to interdigitation with the texture of the tissue surface, but is non-adherent to tissue with which it subsequently comes into contact. The hydrogel consists mostly of polyethylene glycol, imparting good biocompatibility properties, and degrades via hydrolysis into polyethylene glycol, lactic acid and oligomers of acrylic acid[12].

In the current study, we sought to compare polyethylene glycol hydrogel barriers having somewhat similar chemical and physical characteristics but with different modes of chemical cross-linking. Poloxamer 407® has previously been found to significantly reduce adhesion formation in animal models[13–15]. Poloxamer 407 is a copolymer of polyethylene glycol and polypropylene glycol. Because polyethylene glycol is hydrophilic and polypropylene glycol is more hydrophobic, the polymer chains tend to form micellar structures. At low temperatures, a 35% solution of Poloxamer 407 is liquid, but as the solution is warmed to physiological temperatures, the micellar structures aggregate to form a physicochemically cross-linked hydrogel[13]. Thus, Poloxamer 407 can be applied to tissues as an aqueous liquid, with gelation occurring *in situ*. The primary difference between the two hydrogel materials is the nature of their cross-linking, which also results in mechanical differences, the covalently cross-linked hydrogel being stronger. The covalently and physicochemically cross-linked polyethylene glycol-based hydrogels were compared side by side in a rat uterine horn devacularization and serosal injury model of adhesion formation in a blinded and randomized fashion.

MATERIALS AND METHODS

The photopolymerizable precursor (Focal, Inc., Cambridge, MA, USA) was dissolved in (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethane sulphonic acid]) (HEPES) buffered saline (pH 7.4, 10 mM) at a concentration of 10% w/v. Its synthesis and characterization has been described previously[12]. The precursor solution was sterilized by passage through a 0.2 μm filter with a 0.8 μm prefilter. The photoinitiator 2,2-dimethoxy-2-phenyl acetophenone (Aldrich, Milwaukee, WI, USA) was added to the precursor solution at a final concentration of 900 ppm. *N*-Vinyl pyrrolidone (Aldrich) was also present in this preclinical research formulation at a concentration of 0.15%, as it was used as a solvent for the photoinitiator. The precursor solution was used within 2 h of preparation. A 35% solution of Poloxamer 407® (Pluronic F127, BASF, Parsippany, NJ, USA) was prepared in cold HEPES-buffered saline and sterilized by filtration as described above. The Poloxamer 407 solution was stored at 4°C until use.

Female Sprague-Dawley rats (250–300 g) were anaesthetized with pentobarbital (50 mg kg^{-1}). A low midline laparotomy was performed under clean but not sterile conditions. The uterine horns were isolated, and the vasculature was systematically cauterized using bipolar electrocautery, leaving the most distal and most proximal vessels untouched. Two spots on the antimesenteric surface of each horn, centred on the horn and separated by a distance of approximately 2 cm, were cauterized. Following the creation of this injury, the animals were randomly assigned to treatment and control groups, with seven animals per group. In the photopolymerized hydrogel treatment group, 0.5 ml of the precursor solution was applied to each horn and was gelled by exposure to long-wavelength ultraviolet light for 20 s at an irradiance of approximately 10 mW cm^{-2} at a dominant emission of approximately 365 nm. In the Poloxamer 407 treatment group, 2 ml of the cold 35% Poloxamer solution was applied to the uterine horns as well as to other tissues in contact with the uterine horns[14]. In the control group, no treatment was administered following injury. Following treatment, the uterine horns were carefully replaced in the peritoneum, and the musculoperitoneal layer was closed with continuous 4-0 Vicryl sutures (Ethicon, Inc., Somerville, NJ, USA). The skin was closed with 9 mm staples.

On Day 7 after operation, the rats were killed by CO_2 asphyxiation, and the extent and severity of adhesion formation was evaluated by an investigator who was blinded to treatment groupings[10]. The extent of adhesion formation was determined by measuring the total length of the uterine horns as well as the length of the horns involved in adhesions. The extent is reported as the percentage of the uterine horn length involved in adhesions. The severity of adhesion formation was analysed qualitatively. A score of 1 was given to filmy adhesions which were easily separable by hand, while a score of 2 was given to dense adhesions which required sharp instrument dissection for separation. A score of 0 implied that no adhesions were present. Statistical analysis was by the non-parametric Kruskal–Wallis test for the extent of adhesion formation, and by the Chi-squared test for the severity of adhesion formation.

Retention of the barrier materials upon the uterine horns was evaluated in rats without electrocautery injury. Fluorescent polystyrene beads, 1 μm in diameter (Polysciences, Warrington, PA, USA), were added to the photopolymerizable precursor and Poloxamer 407 solutions to aid in the visualization of hydrogel coatings. Female Sprague-Dawley rats (250–300 g) were anaesthetized with pentobarbital (50 mg kg^{-1}). The

uterine horns were isolated and the barriers were applied as described above without injury to the uterine horns. Four animals from each treatment group were killed by CO_2 asphyxiation at each of the following time points: 0, 1, 2, 3 and 5 days. Two tissue samples from each uterine horn were carefully removed, frozen in liquid nitrogen and embedded in Cryoform embedding medium (IEC, Needham, MA, USA). The frozen samples were sectioned to a thickness of 5 μm and mounted on glass sides. The sections were visualized using fluorescence microscopy (Leitz, Rockleigh, NJ, USA), and the thickness of the hydrogel barriers containing fluorescent beads was determined by digital video image processing (Hammamatsu Photonics, Bridgewater, CT, USA).

RESULTS

The efficacy of the covalently and physically cross-linked hydrogel barrier materials is shown in *Table 1*. The covalently cross-linked, photopolymerized hydrogel barrier reduced the extent of adhesion formation by 79% relative to the untreated control group ($P < 0.001$) and reduced the severity of adhesion formation from dense adhesions in the control group to filmy adhesions ($P < 0.001$). The physicochemically cross-linked Poloxamer 407 hydrogel barrier reduced the extent of adhesion formation by 49% relative to the untreated control group ($P < 0.01$), but did not provide a significant reduction in the severity of adhesion formation ($P > 0.7$). The Poloxamer 407 barrier reduced both the extent ($P < 0.03$) and severity ($P < 0.01$) of adhesion formation by a lesser amount than did the photopolymerized hydrogel barrier.

The retention of the covalently and physicochemically cross-linked polyethylene glycol hydrogels upon the rat uterine horn *in vivo* is shown in *Figure 1*. The initial thickness of the covalently cross-linked hydrogel barrier was 176 ± 44 μm (mean $\pm$ s.d.). The covalently cross-linked, photopolymerized hydrogel was present as a continuous barrier through Day 4. On Day 5, the barrier was still present as thin, discontinuous patches with an average thickness of 4.9 ± 0.9 μm. The physicochemically cross-linked Poloxamer 407 hydrogel had an initial thickness of 426 ± 89 μm and was retained upon the uterine horns for less than 2 d *in vivo*. At the end of Day 1, the Poloxamer 407 barrier was continuous and 35 ± 21 μm thick; and at Day 2, no residual barrier was observed.

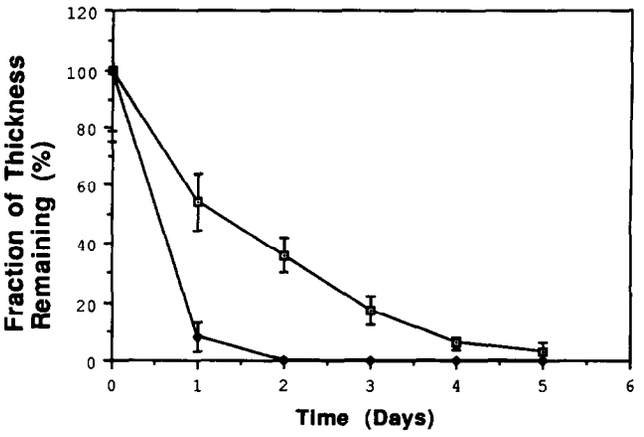

Figure 1 Retention of covalently (open square) and physicochemically (solid square) cross-linked polyethylene glycol-based hydrogels *in vivo* upon the rat uterine horn was evaluated. The thickness of the hydrogel barriers was measured at various time points.

DISCUSSION

The hydrogel adhesion barrier materials compared in this study are similar in many ways, the primary difference between them being the nature of their cross-linking. Their chemical nature and biocompatibility properties are generally similar, both being based on polyethylene glycol. Both hydrogels are applied to tissue in their liquid state with conversion to the hydrogel occurring *in situ* to provide a coating which conformed to the underlying tissue on a cellular length scale. Surprisingly different results were found, however, when the two materials were compared in a rat uterine horn devascularization and serosal injury model of postoperative adhesion formation.

In an attempt to explore the reasons for this difference in efficacy, the retention of the two materials upon the uterine horns was evaluated *in vivo*. The covalently cross-linked, photopolymerized hydrogel was retained for greater than 4 d, while the physically cross-linked hydrogel was retained for less than 2 d. Previous research examining the effect of the duration of intraperitoneal treatment with tissue-type plasminogen activator found that treatment over a 4 d period was ideal, with a 2 d treatment period resulting in significantly greater adhesion formation than a 4 d treatment period[16]. Although it is difficult to compare directly optimal durations of treatment with mechanical versus pharmacological interventions, this differ-

Table 1 Efficacy of covalently and physically cross-linked hydrogels

Group (n)	Extent of adhesion formation (mean% $\pm$ s.d.)	Significance relative to control*	Severity of adhesion formation (0–2)†	Significance relative to control‡
Control (7)	75 ± 10		2,2,2,2,2,2,2	
Covalent (7)	16 ± 6	$P < 0.001$	1,1,1,1,1,1,1	$P < 0.001$
Physical (7)	38 ± 19§	$P < 0.01$	1,2,2,2,2,2,2¶	$P > 0.7$

*By the Kruskal–Wallis test.
†A score of 2 implies dense adhesions, 1 filmy adhesions and 0 no adhesions.
‡By the Chi-squared test.
§$P < 0.03$ for comparison with the covalently cross-linked hydrogel.
¶$P < 0.01$ for comparison with the covalently cross-linked hydrogel.

ence in retention between two materials may be a critical factor in the observed difference in efficacy.

Adhesion of each of these hydrogels to tissue is presumably due to mechanical interlocking with the texture of the underlying tissue. Neither material exhibits adhesion to tissue when implanted as a preformed gel, but instead, both are adhesive only to tissues upon which they are formed. Since both materials are applied to tissues in the liquid state, they may flow into minute crevices on the tissue surface. After the material becomes cross-linked, the mechanical interdigitation results in adhesion between the polymer barrier and the underlying tissue. Other bioadhesive forces, such as van der Waals forces and hydrogen bonding between the tissue and the polymer, probably are not significant in the adhesion of these two polyethylene glycol hydrogels with tissue.

In addition, the increased retention of the covalently cross-linked hydrogel may be facilitated by its stronger mechanical properties. The photopolymerized hydrogel is incapable of flow, having a consistency similar to polyacrylamide gels used for electrophoresis. The Poloxamer 407 hydrogel, however, forms a very loose gel which is easily deformable and capable of flow. The difference in the mechanical strength of the two materials may influence the mechanical interlocking with the underlying tissue.

A number of factors may also cause superior mechanical interlocking of the tissue with the covalently cross-linked hydrogel over the physicochemically cross-linked hydrogel. The viscosity of an adhesive material influences its ability to penetrate the texture of the tissue surface[17]. The 10% precursor solution of the photopolymerized hydrogel has a significantly lower viscosity than the 35% Poloxamer 407 solution, and thus the photopolymerized hydrogel may have better interpenetration with the tissue texture due to better flow into minute crevices. Previous work with cyanoacrylate tissue adhesives found that materials that polymerize very rapidly exhibit lower adhesion than more slowly polymerizing materials, as the rapidly polymerizing materials do not have sufficient time to flow into the tissue texture[18]. This phenomenon may also be influencing the retention of the two hydrogel materials. The Poloxamer 407 gel forms very quickly upon contact with warm tissue, within a few seconds, while the photopolymerized hydrogel is present on the tissue for approximately 10–15 s before exposure to the ultraviolet light, and the cross-linking process occurs over a 20 s period.

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