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Formulation and characterization of poloxamine-based hydrogels as tissue sealants

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ABSTRACT

In situ cross linkable polyethylene glycol (PEG)-based polymers play an increasing role in surgical practice as sealants that provide a barrier to fluid/gas leakage and adhesion formation. This study investigated the gelation behavior and physical properties of hydrogels formed from homogeneous and blended solutions of two acrylated poloxamines (Tetronics® T1107 and T904) of various molecular weights and hydrophilic/lipophilic balances relative to a PEG control. Hydrogels were formed by reverse thermal gelation at physiological temperature (T1107-containing formulations) and covalent crosslinking by Michael-type addition with dithiothreitol. All poloxamine-based hydrogels exhibited thermosensitive behavior and achieved significantly reduced swelling, increased tensile properties and increased tissue bond strength relative to the PEG hydrogel at physiological temperature. Swelling and tensile properties of all poloxamine-based hydrogels were significantly greater at 37 °C relative to 4 °C, suggesting that their improved physical properties derive from cooperative crosslinking by both noncovalent and covalent mechanisms. Poloxamine-based hydrogels were cytocompatible and underwent hydrolytic degradation over 2–5 weeks, depending on their T1107/T904 composition. In conclusion, select poloxamine-based hydrogels possess a number of properties potentially beneficial to tissue sealant applications, including a substantial increase in viscosity between room/physiological temperatures, resistance to cell adhesion and maintenance of a stable volume during equilibration.

1. Introduction

Sutures and surgical staples are the most widely used methods of wound closure, despite limitations, including the requirement for anesthesia; risk of infection; time and skill required; and difficulty in application/retention in soft tissues such as internal organs [1,2]. This has generated considerable interest in the development of in situ curable polymeric materials that may be used as tissue adhesives/sealants either alone or as an adjunct to sutures. One major group of these materials are tissue glues based on naturally derived materials such as fibrin glue, gelatin–resorcinol–formaldehyde glue and, more recently, BioGlue® (CryoLife) composed of bovine serum albumin crosslinked by glutaraldehyde. Fibrin and BioGlue® are FDA approved and have been used successfully in many clinical applications [3–5]. Although advantageous because of their intrinsic biodegradability, naturally derived materials involve risks of possible viral transmission, hypersensitive reactions to bovine proteins and relatively low mechanical properties. In addition, cytoxicity has been reported with the use of low molecular weight aldehyde-based crosslinkers [6]. To overcome these limitations, recent studies have focused on the development of fully synthetic alternatives.

Alkyl-2-cyanoacrylates were the first materials investigated as synthetic tissue adhesives. These low molecular weight monomers undergo rapid polymerization upon exposure to weak bases present in tissue. Cyanoacrylates with short side chains (methyl/ethyl) were first studied, but deemed unsuitable due to high stiffness and evidence of cytotoxicity and tissue necrosis [7,8]. Butyl (Indermil®, Covidien and Histoacryl®, Tissueseal) and octyl (Dermabond®, Closure Medical) derivatives have been approved for topical wound closure and as bacterial barriers [9–11]. However, internal use of cyanoacrylates remains limited due to their relatively slow degradation and concerns about toxicity of the degradation products. Recent developments in materials for internal applications have focused on various polyethylene glycol (PEG)-based hydrogels such as FocalSeal® (Genzyme BioSurgery), CoSeal® (Baxter) and DuraSeal™ (Confluent Surgical). FocalSeal® consists of PEG diols modified with hydrolytically degradable ester linkages and terminal acrylates for photoinitiated polymerization [12]. It has been successfully used for sealing lung air leaks, but its widespread adoption has been hindered by relatively slow curing and the additional surgical equipment required [13,14]. CoSeal® and DuraSeal™ are both crosslinked by step-growth polymerizations of 4-arm PEG-based macromonomers with degradable linkages and terminal reactive groups. CoSeal® and Duraseal™ are FDA

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approved for vascular and dural sealing, respectively, and have shown efficacy in other experimental models as well [15–17]. A common challenge to PEG-based sealants is significant post-polymerization swelling, resulting in increases in volume ranging from 50 to 400% relative to the initial volume dispensed [18–20]. Two recent reports have described serious clinical complications believed to be attributable to swelling-induced compression of neighboring tissues [21,22].

The objective of this study was to develop and characterize hydrogels based on acrylated poloxamine (Tetronic®) block copolymers. Poloxamines are a family of 4-arm polypropylene oxide (PPO)–polyethylene oxide (PEO) block copolymers that spontaneously form micelles in aqueous solution consisting of a hydrophobic PPO core domain surrounded by a hydrophilic PEO shell [23]. They have recently been investigated for applications in drug delivery, gene therapy and tissue engineering [24–29]. Cellesi and co-workers first described the fabrication of poloxamine microspheres for cell encapsulation based on a "tandem gelation" process combining reverse thermal gelation and covalent crosslinking [30,31]. We hypothesized that the combination of noncovalent and covalent crosslinking available with these materials would provide improved physical properties for sealant applications relative to conventional PEG-based hydrogels prepared solely by covalent crosslinking. In this study, hydrogels were crosslinked from homogeneous and blended solutions of two poloxamines (T1107 and T904) of different molecular weights and hydrophilic/lipophilic balances (HLBs). All poloxamine-based hydrogels exhibited thermosensitive behavior and at physiological temperature achieved significantly lower swelling and significantly higher tensile properties and tissue bond strength relative to the PEG hydrogel control.

2. Materials and methods

2.1. Materials

Tetronics® T1107 (T1107, MW: 15 kDa, HLB: 18-23) and T904 (T904, MW: 6.7 kDa, HLB: 12-18) were kindly donated by BASF corporation (USA). Acrylated 4-arm polyethylene glycol (PEG; MW: 10 kDa), prepared as previously described [32], was a gift from Dr. Andrew Metters (Clemson University). Acryloyl chloride, Celite fine 500 and 4-methoxyphenol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Toluene (HPLC grade), ethyl ether (anhydrous, BHT stabilized), hexanes (HPLC grade) and anhydrous sodium sulfate were purchased from Fisher Scientific (NJ, USA). Dichloromethane (HPLC grade), triethylamine (TEA), dithiothreitol (DTT), sodium bicarbonate, calcium hydride and $CDCl_3$ were obtained from Acros Organics (NJ, USA). Dichloromethane was dried with calcium hydride and stored over molecular sieves (Grade 514, Type 4A). All other chemicals were used as received.

2.2. Preparation and characterization of acrylated poloxamines

2.2.1. Preparation of acrylated T1107 (T1107 ACR)

T1107 ACR was prepared by reaction of the T1107 terminal hydroxyl groups with acryloyl chloride. All glassware was dried in a vacuum oven and fully purged with argon before use. A 15 g (1 mmol) quantity of T1107 was dehydrated by azeotropic distillation with toluene for 2 h and the toluene was then removed by rotary evaporation (Buchi Rotavapor®, Switzerland). After cooling to room temperature, the dried T1107 was dissolved in 120 ml of dry dichloromethane in a 250 ml round-bottom flask under an argon atmosphere. Once the T1107 was completely dissolved, TEA (4 mmol) was added and the reaction flask cooled on an ice bath. Acryloyl chloride (8 mmol) in 30 ml of dry dichloromethane was added dropwise with an addition funnel over 2 h. The ice bath was removed after 2 h and the reaction was allowed to continue at room temperature for 24 h. The reactant was filtered through Celite to remove TEA-HCl salt, then concentrated by rotary evaporation to reduce the solvent to one-tenth of its initial volume. The residue was precipitated in 500 ml of cold ethyl ether, recovered by filtration and dried under vacuum for a few hours. The product was redissolved in 150 ml of dichloromethane, washed with 15 ml of 10% w/v sodium bicarbonate solution until the pH of the solution was neutral, followed by water washes (15 ml each) until the pH of the water was neutral, and then dried with anhydrous sodium sulfate. The solution was concentrated by rotary evaporation, the residue precipitated in ethyl ether (−20 °C), and washed three times with cold ethyl ether. The final product was recovered by filtration, dried for 48 h in a vacuum desiccator and stored at 4 °C.

2.2.2. Preparation of acrylated T904 (T904 ACR)

T904 was dried and reacted with acryloyl chloride in the presence of TEA as described above for 24 h at 4 °C. The purification procedure was adjusted to account for the reduced thermal stability and increased ether solubility of acrylated T904. Briefly, after filtration and solvent reduction (in the presence of a small amount of 4-methoxyphenol), the residue was precipitated in 50:50 ethyl ether:hexane overnight at −20 °C. After decantation of the supernatant, the product was redissolved in dichloromethane, washed as described above, concentrated and precipitated once in 50:50 ethyl ether:hexane and recrystallized twice from ice cold ethyl ether after 48 h at 4 °C. The product was dried in a vacuum dessicator at 4 °C and stored at −20 °C.

2.2.2.1. ^{1}H-NMR ($CDCl_3$) characterization. T1107 ACR and T904 ACR: δ = 1.1 (m, PPO CH_3), 3.4 (m, PPO CH), 3.54 (m, PPO CH_2), 3.65 (m, PEO CH_2), 4.32 (t, -$CCH_2OC(=O)$-), 5.8 and 6.4 (d, 2H, acrylic –CH_2), 6.15 (q, 1H, acrylic –CH) ppm. A representative NMR spectra of acrylated T1107 is shown in Fig. 1. The percent acrylation efficiency was calculated based on the ratio of the integrals of the PEG backbone (δ = 3.5∼3.7) and the acrylate peaks (δ = 6.15–6.4). Acrylation efficiencies of 94–100% (T1107 ACR) and 84–90% (T904 ACR) were consistently obtained. The ratio of acrylate to activated PEG terminal methylene (δ = 4.32) peaks closely approximated the 3:2 theoretical value, confirming the efficient removal of unreacted acryloyl chloride.

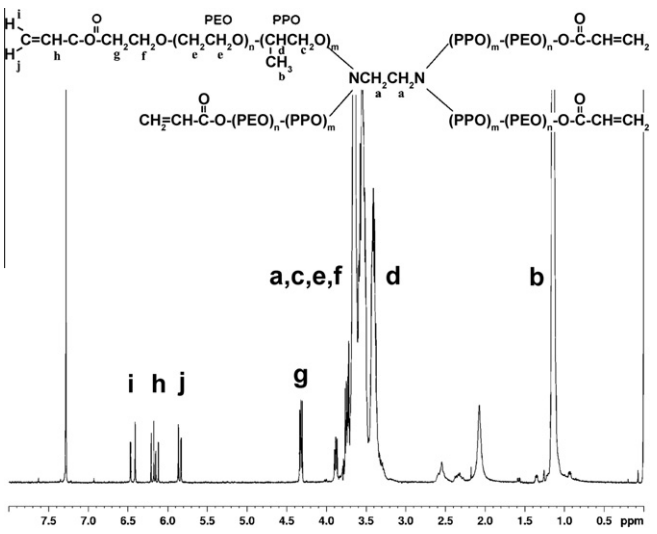

Fig. 1. Poloxamine (Tetronic®) structure (T1107: $n = 60$, $m = 19$; T904: $n = 15$, $m = 17$) and representative ^{1}H-NMR spectrum of acrylated T1107.