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Strong poly(ethylene oxide) based gel adhesives via oxime cross-linking

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ABSTRACT

There is a demand for materials to replace or augment the use of sutures and staples in surgical procedures. Currently available commercial surgical adhesives provide either high bond strength with biological toxicity or polymer and protein-based products that are biologically acceptable (though with potential sensitizing potential) but have much reduced bond strength. It is desirable to provide novel biocompatible and biodegradable surgical adhesives/sealants capable of high strength with minimal immune or inflammatory response. In this work, we report the end group derivatization of 8-arm star PEOs with aldehyde and amine end groups. Gels were prepared employing the Schiff-base chemistry between the aldehydes and the amines. Gel setting times, swelling behavior and rheological characterization were carried out for these gels. The mechanical-viscoelastic properties were found to be directly proportional to the crosslinking density of the gels, the 10K PEO gel was stiffer in comparison to the 20K PEO gel. The adhesive properties of these gels were tested using porcine skin and showed excellent adhesion properties. Cytotoxicity studies were carried out for the individual gel components using two different methods: (a) Crystal Violet Staining assay (CVS assay) and (b) impedance and cell index measurement by the xCELLigence system at concentrations >5%. Gels prepared by mixing 20% w/w solutions were also tested for cytotoxicity. The results revealed that the individual gel components as well as the prepared gels and their leachables were non-cytotoxic at these concentrations.

Statement of Significance

This work presents a new type of glue that is aimed at surgery applications using a water soluble star shaped polymer. It show excellent adhesion to skin and is tough and easy to use. We show that it is very biocompatible based on tests on live human cells, and could therefore in principle be used for internal surgery. Comparison with other reported and commercial glues shows that it is stronger than most, and does not swell in water to the same degree as many other water based bioadhesives.

1. Introduction

In recent years there has been an increased interest in replacing sutures and staples used in surgical procedures with adhesive bonds and synthetic sealants. The reasons for this are due to improvements over the above mechanical procedures such as the ability of the adhesive to hold the tissue firmly with minimal blood leaks, no trauma or harm to the surrounding tissue and no adverse side effects of the glue or any of its by-products. Conventional wound closure procedures have several potential limitations. For example, fluid leakage between the sutures or staples and through the cuts or holes created by these sutures and staples can further lead to other complications and infections [1–3].

Biomedical researchers have attempted to develop various sealants and adhesives to bind tissue, and/or tissue to prosthetic devices, in a wide range of surgical procedures [4,5]. An example of a naturally-derived adhesive is based on fibrin. Despite its usefulness, the major limitations of the fibrin based sealant are its complex preparation procedure, as well as its slow cure and low strength [6–9]. There are a number of biologically-based two-component surgical adhesives including gelatin/resorcinol/formaldehyde (GRF) and albumin/glutaraldehyde glues. Regardless of their efficacy to bind tissue, wide spread acceptance of the GRF adhesives has been limited because of their cytotoxicity from the release of formaldehyde [10]. Polyethylene oxide (PEO) materials have been widely studied, including chemically crosslinked PEOs and photopolymerizable multi-arm PEOs. The major drawbacks of these PEO-based sealants are their high degree of swelling and low adhesive and mechanical strength as compared to the

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cyanoacrylate glues [11,12]. Although cyanoacrylate based tissue adhesives have wide applications in medical fields, they have several disadvantages due to their low biocompatibility. The major drawbacks of cyanoacrylates include their low viscosity, as well as the high modulus of the cured glue which can lead to adhesive joint failure and/or irritation of the surrounding tissue and its necrosis [13–15]. This limits their use to surface wounds only.

The aim of this study is to provide a surgical adhesive with good tensile strength, adhesive properties, and biocompatibility. PEO was chosen due to its chemical stability, water solubility, non-toxicity, and biocompatibility [16–20]. There are several PEO-based glues/adhesives currently in use for numerous medical/surgical purposes, such as FocalSeal® (Genzyme BioSurgery), CoSeal® (Baxter) and DuraSeal™ (Confluent Surgical). FocalSeal® (formed by photo-crosslinking of PEO-derivatives) has been successfully used in internal surgical procedures, but its use is hindered mainly due to the slow curing rate and the additional surgical equipment required [21]. CoSeal® and DuraSeal™ are both crosslinked by step-growth polymerizations of multi-arm PEG-based macromonomers. Both these gels are FDA approved and are effectively used for vascular and dural sealing, but have restricted use due to their high degree of swelling [22–25]. Thus, there is a demand for an adhesive which will have improved properties such as optimal setting times, less swelling, better strength with good flexibility, along with ease of dispensing [12,26]. As far as we can determine this is the first study of bioadhesives that uses oxime chemistry in the cross-linking reaction. The main advantage of using oximes is that it allows the use of more stable and less cytotoxic benzaldehyde derivatives, as opposed to the alkyl aldehydes normally used in imine cross-linking.

2. Materials and methods

2.1. Materials

Poly(ethylene oxide) 8-arm (PEO-OH), average molecular weight ($M_n \sim$ 10,000, 20,000 and 40,000) were purchased from JenKem Technology (USA). Dulbecco's modified eagle medium, (DMEM, Gibco®) was purchased from (Invitrogen, USA). All other reagents and solvents were procured from Acros Organics and Sigma–Aldrich and used without further purification.

2.2. Synthesis and characterization of 8-arm star poly(ethylene oxide) derivatives

Synthesis and analytical data for all PEO derivatives: PEO_8-aldehyde (PEO_8-CHO; **2a-c**), PEO_8-chloride (PEO_8-Cl; **3a-c**), PEO8-azide (PEO_8-N_3; **4a-c**) and PEO8-amine (PEO_8-NH_2; **5a-c**) are summarized in the Supporting information (S1–S4).

2.2.1. Synthesis of PEO_8-benzaldehyde (PEO_8-PhCHO) (**6a–c**)

PEO_8-PhCHO **6** was synthesized in two steps following a procedure reported for linear PEO polymers [27]. Briefly, PEO_8-OH was first converted to its chloride derivative PEO_8-Cl (**3a–c**). Further reaction of PEO_8-Cl with 4-hydroxybenzaldehyde in presence of a base yielded the desired PEO_8-PhCHO (**6a–c**) as a pale yellow waxy solid. Detailed procedure is given in Supplementary information (S5). The extent of conversion was calculated from the ratio of integration of the benzaldehyde and methyl peaks at 9.88, 4.21 and 3.88 δ ppm, respectively, to the PEO_8-backbone peaks observed between 3.82 and 3.38 δ ppm in ^{1}H NMR spectrum (Fig. S1a). The formation of PEO-benzaldehyde was also confirmed from the ^{13}C NMR spectrum from the observation of the carbonyl peak of the benzaldehyde 190 δ ppm (Fig. S1b).

2.2.2. Synthesis of PEO_8-oxyamine (PEO_8-ONH_2) (**8a–c**)

PEO_8-ONH_2 **8** was synthesized by a two-step process, following a reported procedure [28]. The conversion of PEO_8-OH to its corresponding PEO_8-phthalimide **7** (PEO_8-ONHPI) derivative (compounds **7a–c**, $M_n \sim$ 10K, 20K and 40K, respectively) was performed by a Mitsunobu reaction (S6). PEO_8-ONHPI derivatives **7a–c** were then hydrazynolysed in the presence of excess hydrazine hydrate to yield the desired reactive oxyamine derivatives PEO_8-ONH_2 (**8a–c**). Formation of the intermediate PEO_8-ONHPI as well as the PEO_8-ONH_2 was established by ^{1}H and ^{13}C spectroscopy (Fig. S2–S4). Formation of the PEO_8-ONH_2 was confirmed by the disappearance of the peak at δ 164 ppm corresponding to the carbonyl of the phthalimide group in the ^{13}C NMR spectrum of PEO_8-ONH_2 (Fig. S4). In addition, a new peak at δ 62 ppm was observed in the ^{13}C NMR spectrum, which is the characteristic peak for the carbon attached to the –aminoxy group PEO–$CH_2\underline{C}H_2$–ONH_2 suggesting quantitative conversion of **7** to the corresponding oxyamine derivative **8**. The analysis of the ^{1}H NMR spectrum of **7** revealed the disappearance of the peaks corresponding to the phthalimide group in the aromatic region as well as the signal for the methylene group protons attached to the phthalimide group (PEO-$OCH_2C\underline{H}_2$-ONHPI) at δ 4.37 ppm. The disappearance of these two peaks was the only confirmation for the formation of the amine derivative **8**, and no distinct signal for the methylene protons adjacent to the aminoxy end groups (PEO-$OCH_2C\underline{H}_2$-ONH_2) could be observed for comparison in the ^{1}H NMR spectrum (Fig. S3b). Hence, it was decided to record the ^{1}H NMR spectrum in acetone-d_6 [29]. Acetone-d_6 not only acts as the NMR solvent but also as a derivatizing reagent. The ketone reacts with the –aminoxy end groups to form a ketoxime ether conjugate (Fig. S3c).

2.3. Gel setting times and swelling behavior

The gels were prepared by mixing solutions of PEO_8-PhCHO **6** (33 mol.% excess) and the PEO_8-ONH_2 **8** derivatives in 1:0.75 M ratios (total solids 50% w/w in water). Further studies were carried out using this set of gels: **G-10**: ($M_n \sim$ 10,000) PEO_8-PhCHO + PEO_8-ONH_2 and **Gel-20**: ($M_n \sim$ 20,000) PEO_8-PhCHO + PEO_8-ONH_2. Analogous PEO-derivatives with $M_n \sim$ 40,000 were prepared but did not gel. The cross-over point between the storage (G') and loss (G'') moduli obtained from the rheological measurements was used to determine the gelation time.

Swelling measurements were carried out at room temperature. Gels were made as mentioned above. The pre-weighed dry gel sample was immersed in a vial filled with water. The change in weight of the gel was measured at regular intervals by removing the gel from the vial, wiping away excess water, and then weighing it. The gel was re-immersed in water. The weight change was recorded until no further change in the weight was observed for three successive measurements (Fig. S5). The swelling ratios were calculated as follows:

$$\text{Swelling ration (wt.\%)} = \frac{W_f - W_i}{W_i} \times 100$$

where W_f is the maximal gel mass measured throughout the suspension period and W_i is the initial mass prior to suspension (dry sample).

2.4. Crosslink density calculations

The crosslink density can be calculated from the concentration of the limiting reactant **A** (in our case the oxyamine) for a two component reaction. If the concentration of **A** is [**A**], and the number of