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Strong tissue glue with tunable elasticity

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ABSTRACT

Many bio-adhesive materials adhere weakly to tissue due to their high water content and weak structural integrity. Others provide desirable adhesive strength but suffer from rigid structure and lack of elasticity after administration. We have developed two water-free, liquid four-armed PEG pre-polymers modified with NHS or with NH_2 end groups which upon mixing changed from liquids to an elastic solid. The sealant and adhesive properties increased with the amount of the %v/v PEG_4-NHS pre-polymer, and achieved adhesive properties comparable to those of cyanoacrylate glues. All mixtures showed minimal cytotoxicity *in vitro*. Mixtures of 90%v/v PEG_4-NHS were retained in the subcutaneous space *in vivo* for up to 14 days with minimal inflammation. This material's combination of desirable mechanical properties and biocompatibility has potential in numerous biomedical applications.

Statement of Significance

Many bio-adhesive materials adhere weakly to tissue (e.g. hydrogels) due to their high water content and weak structural integrity. Others provide desirable mechanical properties but suffer from poor biocompatibility (e.g. cyanoacrylates). This study proposes a new concept for the formation of super strong and tunable tissue glues. Our bio-materials' enhanced performance is the product of new neat (without water or other solvents) liquid polymers that solidify after administration while allowing interactions with the tissue. Moreover, the elastic modulus of these materials could easily be tuned without compromising biocompatibility. This system could be an attractive alternative to sutures and staples since it can be applied more quickly, causes less pain and may require less equipment while maintaining the desired adhesion strength.

1. Introduction

Bio-adhesive materials are important in many fields of medicine, for tissue reconstruction, adhesion to mucosal tissues, tissue sealing, and drug delivery. Tissue adhesives may provide attractive alternatives to sutures and staples since they can be applied more quickly, cause less pain, and obviate the need for suture removal [1–4]. Although various bio-adhesive formulations have been developed for soft tissues, their clinical use is still limited by weak mechanical properties [5]. For example, fibrin based tissue glues, composed of purified fibrinogen and thrombin, adhere very weakly to tissues and therefore their clinical use require the support of sutures or staples [6]. Synthetic polymers have also been suggested as tissue adhesives, including two-component PEGs (CoSeal®), a mixture of PEG ester and trilysine (DuraSeal®) and light activated PEG (FocalSeal®). However, these synthetic adhesives have showed comparable strength to fibrin sealant, attributable to their high water content, between 90% and 99% **[7]**. New hydrogels with better mechanical properties have been suggested including hydrogels with double network [8] or covalently cross-linked matrixes with self-healing properties [9]. However, while these developments strengthen the cohesive force of the hydrogels, their adhesion properties remain weak due to the low density of reactive end groups. As a result, currently available hydrogels act like sealants – they cover wounds rather than close them [10]. Cyanoacrylate derivatives, on the other hand, adhere very strongly to tissues,

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but are limited only to external surfaces such as skin and eyes due to their exothermic nature of reaction and by toxic products such as cyanoacetate and formaldehyde [11]. In addition, polymerized cyanoacrylates tend to form a rigid structure that may lose integrity in dynamic conditions and may interfere with the natural healing process [12,13]. Therefore, cyanoacrylate formulations are contraindicated in tissues that undergo large deformations (e.g. meniscus) or to skin tissue subjected to increased tension such as elbows and knee [14]. Hence, new tissue adhesives with excellent adhesion strength and elasticity are desired.

In our previous work we developed an injectable material compose of water-free, liquid four-armed PEG modified with dopamine end groups [15]. This material changed from liquid to a non-adhesive stiff solid by reaction with a small volume of ferric (Fe^{+3}) aqueous solution. Encouraged by the excellent mechanical properties of the cross-linked PEG_4-dopamine, we have hypothesized that a novel bio-adhesive systems could be achieved by utilizing a completely neat (containing no water as a solvent) low molecular weight polymeric system that will also be modified with functional groups that promote cross-linking and adhesiveness to tissues. We expected the adhesive strength of such an elastic material to be relatively higher than that of existing hydrogels since it would have a greater density of reactive end groups compared to polymers of higher molecular weights and since it would not contain any solvent. Our system (Fig. 1) employs two complementary neat liquid pre-polymers based on four-armed polyethylene glycol (PEG_4: 2000 Da). This polymer was chosen since it has low immunogenicity and toxicity [16,17] and since it is liquid and flowable at room temperature [18]. One prepolymer was formed by functionalizing with amine groups (PEG_4-NH_2), the other with N-hydroxysuccinimide (NHS)-esters (PEG_4-NHS) (Fig. S1). The reaction between amine and NHS esters yields a stable amide bond [19]. The NHS groups on PEG_4-NHS were intended to react with amine groups from PEG_4-NH_2, providing cohesive force within the adhesive, and amine groups from adjacent tissue, to provide adhesive force [3]. The formed amide bonds can ultimately undergo hydrolytic degradation [19]. However, given the hydrophilic nature of PEG and the ability of similar systems to absorbed water and considerably swell [15], the hydrolytic degradation is likely to occur only after small fragments of the fully swollen gels erode from surface. We note that short, liquid biocompatible polymers with activated acid functional groups have been suggested as cross-linking agents for biological fluids such as blood, plasma and serum [20].

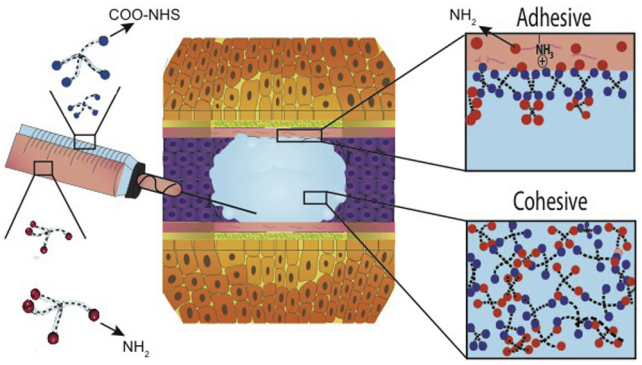

Fig. 1. Schema of the suggested bio-adhesive: The bio-adhesive consists of two complementary neat pre-polymers that cross-link while mixing during administration. For adhesive forces, NHS groups (blue circles) are able to covalently bind NH_2 groups (red circles) presented on the tissue. For cohesive forces, NHS groups (blue circles) are able to covalently bind NH_2 groups (red circles) of the complementary pre-polymers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Chemicals

Phthalimide, triphenylphosphine, Diisopropyl azodicarboxylate, HCl, NaOH, hydrazine monohydrate, N,N-disuccinimidyl glutarate and 4-(dimethyl amino) pyridine phosphate Dry acetone, methanol and ethanol were purchased from Sigma-Aldrich, Inc. (St Louis, MO). Four-armed PEG was purchased from Jensen USA (TX, USA). Methylene chloride, tetrahydrofuran (THF), dimethylformamide (DMF), diethyl ether and Deuterium oxide were purchased from Cambridge Isotope Laboratories, Inc. (Cambridge, MA).

2.2. Synthesis

PEG_4-NH_2 was synthesized by reacting PEG_4 with phthalimide, then with hydrazine monohydrate [21] (Fig. S2). In brief, PEG_4 (2 mmol), phthalimide (16 mmol) and triphenylphosphine (16 mmol) were dissolved in 40 mL tetrahydrofuran (THF). Diisopropyl azodicarboxylate (DIAD, 16 mmol) in 20 mL THF was added dropwise and the solution was allowed to stir for 72 h. Then, THF was evaporated by rotary evaporation and the material obtained was dissolved in ethanol (100 ml) and treated with hydrazine monohydrate (0.1 mol) under reflux for 24 h. The resulting suspension was filtered and the solvent was removed by rotary evaporation. Repeated dissolutions in 2 mL methylene chloride followed by precipitations in 4 °C diethyl ether were performed until a homogenous yellowish product was achieved. 92% of the PEG end groups were functionalized with NHS as confirmed by ^{1}H NMR (Fig. S2 in the Supporting Information).

PEG_4-NHS was synthesized by reacting 4 gr PEG_4, 3 g N,N-disuccinimidyl glutarate and 1.5 g 4-(dimethyl amino) pyridine in 80 DMF (Fig. S1). After 6 h, DMF was evaporated by rotary evaporation and the formed PEG_4-NHS was dissolved in 2 mL methylene chloride and precipitated in 4 °C diethyl ether. The polymer was then re-dissolved in 1 mL methylene chloride and precipitated again in 4 °C diethyl ether. 92% of the PEG end groups were functionalized with NHS as confirmed by ^{1}H NMR (Fig. S3 in the Supporting Information).

2.3. Chemical characterization

The chemical structures of the molecule as well as the efficiency of the modification were determined by ^{1}H NMR spectroscopy using a Varian Mercury (Palo Alto, CA) 300 MHz spectrometer in D_2O (Fig. S2 and S3 in the Supporting Information). The amine content was determined by comparing the integral value of pentaerythritol methylene protons at $\delta = 3.5$ to the hydroxyl protons of the amine at $\delta = 2.62–2.66$ (Fig. S2). The NHS content was determined by comparing the integral value of pentaerythritol methylene protons at $\delta = 3.6$ to the NHS protons at $\delta = 2.8$ (Fig. S3).

2.4. Mechanical properties

2.4.1. Rheology

Rheological experiments were performed on Discovery Hybrid Rheometer (DHR-2, TA Instruments, DE, USA) using a parallel 8 mm diameter plate, to evaluate the rheological profiles of the cross-linking presses. The elastomer sample was placed between parallel plates at 37 °C and the gap between the plates were set to 0.5 mm. Excess sample was trimmed off the lower plate. The instrument was controlled using the Trios program (TA Instruments, DE, USA). Shear storage modulus (G') was measured by performing a shear frequency-sweep test in a range of frequencies between 1 and 10 Hz at a constant shear strain of 0.35%. The results