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Phloroglucinol-based biomimetic adhesives for medical applications

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Abstract

An adhesive that functions well under moist conditions could facilitate many surgical procedures. In recent studies we designed novel biomimetic glues which mimic the adhesion mechanism of algae, renowned for their remarkable adherence to wet surfaces. Here we extend our previous studies and propose biomimetic formulations, composed of alginate gel and native phloroglucinol, that do not induce cell cytotoxicity. Characterization of the adherence to tissues showed that adhesion was directly related to the mechanical strength of the cross-linked alginate. Therefore the adhesion strength can be altered by changing the source of the calcium cross-linker, the alginate G-content or the molecular weight of the alginate. The adhesion strength was comparable to that of Tisseel™, a commercial tissue adhesive.

Keywords: Biomimetic; Tissue adhesive; Alginate; Phloroglucinol; Biocompatibility

1. Introduction

Although widely applied and well established in the clinic, traditional tissue reattachment methods, such as sutures, staples and wires, do not meet all of the surgeon's needs. These methods are not suitable for inherently complicated procedures, such as stopping leaks of bodily fluids and air in blood vessels and friable tissues such as lung, liver, spleen and kidney. Therefore, an interest in materials that can supplement the traditional incision closure in surgical procedures has emerged. Tissue adhesives that are developed for these applications should first and foremost attach themselves to a wet surface at body temperature, and should also be non-toxic, biocompatible and biodegradable [1].

Currently there are several types of commercially available tissue adhesives, which are traditionally classified into two categories: synthetic and natural. Synthetic adhesives include cyanoacrylate glues, which are marketed as liquid monomers which rapidly polymerize in the presence of moisture. These glues create a strong yet flexible bond, but can only be used externally because they cause an intense inflammatory response and are toxic when making contact with noncutaneous surfaces [2]. Another synthetic adhesive is based on a polymeric hydrogel made from poly(ethylene glycol) (PEG). This product is used both as a fluid barrier and as a hemostatic agent, and is biodegraded within 1–6 weeks [3]. Although it has been claimed that PEG-based adhesives are effective in sealing suture line bleeding [3], no quantitative data supporting this claim could be found in the relevant scientific literature. Tissue adhesives based on natural polymers, cross-linked via biochemical reactions, offer a more biocompatible alternative to synthetic glues. Fibrin sealants are made from a number of components produced from pooled human plasma that

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enables the adhesive to mimic the final stages of blood clotting [4]. Concerns related to possible contamination of blood products with viruses such as HIV have led to the development of virus-inactivated fibrin sealants. These sealants offer an excellent biocompatibility and low toxicity, yet have a complex preparation procedure, slow curing and rather poor bonding strength [3]. Adhesives based on either gelatin or bovine serum albumin (BSA) are widely used for hemostasis in all types of surgery. Despite their relatively high bonding strength, technical problems and toxicity of some components have limited their application in the clinic [5,6]. Thus, although significant developments have been made in the area of medical adhesives over the last 20 years, none of the currently available tissue adhesives are widely used. Synthetic or semi-synthetic adhesives suffer from low biocompatibility, potential toxicity and low adherence to wet surfaces. Biological glues, on the other hand, are expensive, often exhibit relatively poor mechanical and tissue-bonding properties, and are potentially pro-inflammatory because most of them are based on proteins. Therefore, new materials need to be developed in order to meet the increasing clinical demand for more effective surgical adhesives.

One possible route to developing such an adhesive is through a biomimetic approach, which encompasses artificial materials that mimic natural forms [7]. In the past few years our group has studied natural materials extracted from the brown alga *Fucus serratus*. Like other marine organisms, algae produce and secrete adhesives that form permanent, strong and flexible underwater bonds to a variety of substrates. Our studies have shown that the adhesives produced by *F. serratus* are multicomponent polymeric materials composed of polyphenol and alginate, which is cross-linked by divalent calcium ions [8,9]. Following the biomimetic approach, the natural polyphenol was replaced with its synthetic monomer unit, phloroglucinol. The adherence capabilities of the biomimetic glue to a variety of substrates were of the same order of magnitude as those reconstructed from the alga [7]. The feasibility of utilizing this biomimetic glue for medical applications was demonstrated using tensile assays with porcine muscle tissues as a model surface. In the current study, we further explored the properties of the biomimetic glues. Since adherence to tissues is the most significant property of a medical adhesive, we explored several modifications that may be used to alter this property. Additionally, preliminary evaluations of the glue's cytotoxicity, biocompatibility and shelf life were performed.

2. Materials and methods

2.1. Materials

D(+)-Gluconic acid δ-lactone (GDL) and ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were purchased from Fluka. Phloroglucinol ($C_6H_6O_3$), calcium carbonate, bromoperoxidase (BPO) and alginate lyase from Flavobacterium sp (A1603) with an activity of 10,000 units per gram of solid were purchased from Sigma. NaOH was purchased from Bio Lab, and $CaCl_2$ from J.T. Baker. Alginate (LF 200S, HF120RBS) was supplied by FMC Biopolymers (Drammen, Norway). Hydrogen peroxide (H_2O_2), 30 wt.%, was purchased from Merck. Potassium iodide (KI) was purchased from Spectrum Chemical Mfg. Corp. Mylar (a trademark of DuPont for polyester) was obtained from Pronat Company (Netanya, Israel). Porcine muscle tissues were donated by the "White Meilia" slaughterhouse (Meilia, Israel). Prior to testing, the tissue specimens were stored at 4 °C in Krebs solution treated with gentamicin antibiotic [10]. The commercial fibrin sealant Tisseel™ (Baxter Health Corp. Deerfield, IL) was used for comparison.

2.2. Adhesive preparation

Adhesive formulations were prepared by dissolving a mixture of phloroglucinol, alginate and calcium ions in mili-Q water. Calcium ions were added either by blending the mixture with the insoluble salt $CaCO_3$ or with Ca-EGTA solution. Next, the glues were cured by addition of the slowly hydrolyzing acid GDL. The preparation of Ca-EGTA solution has been described elsewhere [11]. Briefly, an equal molar amount of $CaCl_2$ and EGTA was dissolved in water and the pH was adjusted to 7 by adding 1 M NaOH. Unless otherwise stated, a composition containing 5 mg ml^{-1} phloroglucinol, 25 mg ml^{-1} alginate and 5.5 mM calcium ions was used. For the oxidized adhesive, a composition containing 5 mg ml^{-1} phloroglucinol, 0.75 U ml^{-1} BPO, 0.44% H_2O_2, 4.4 mg ml^{-1} KI, 15 mg ml^{-1} alginate and 4 mM calcium ions was used.

2.3. Adhesion properties

Characterization of the adhesion properties was performed using uniaxial tensile testing [12]. Specimens were prepared by attaching two Mylar™ rectangular strips, having an edge size of 20 or 25 mm, to stainless steel sample holders using commercially available cyanoacrylate glue. When porcine tissue was used as the adherent, a thin slice of tissue was attached to each of the Mylar sheets using cyanoacrylate glue. A volume of 0.07 μl of adhesive per mm^2 of glued area was then applied onto one surface and immediately covered with the second tissue specimen, producing an adhesive layer with thickness of ~0.07 mm. The overlapped samples were immediately clamped together for the desired period of time to prevent relative motion and allow the adhesive to set. Preliminary tests showed that after a period of 20 min the tensile strength reaches a plateau. Therefore, uniaxial tensile testing was performed 20 min after preparation using a Lloyd uniaxial testing device equipped with a 50 N load cell. The force necessary to separate the two adherent strips was determined at a crosshead speed of 5 mm min^{-1}. Since none of the stress–strain curves displayed a yield point, the maximum tensile