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Effect of alkyl chain length on the interfacial strength of surgical sealants composed of hydrophobically-modified Alaska-pollock-derived gelatins and poly(ethylene)glycol-based four-armed crosslinker

Ryo Mizuta[a,b], Temmei Ito[a,b], Tetsushi Taguchi[a,b,*]

[a] Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan
[b] Polymeric Biomaterials Group, Biomaterials Field, Research Center for Functional Materials, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

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ABSTRACT

Surgical sealants are widely used clinically. Fibrin sealant is a commonly used sealant, but is ineffective under wet conditions during surgery. In this study, we developed surgical sealants composed of hydrophobically modified Alaska-pollock-derived gelatins (hm-ApGltns) with different alkyl chain lengths from C3 to C18 and a poly(ethylene)glycol-based 4-armed crosslinker (4S-PEG). The burst strength of the hm-ApGltns-based sealant was evaluated using a fresh porcine blood vessel and was found to increase with increasing alkyl chain length from 167 ± 22 to 299 ± 43 mmHg when the substitution ratio of amino groups of ApGltn was around 10 mol%. The maximum burst strength was observed when stearoyl-group modified ApGltn (Ste-ApGltn)/4S-PEG-based sealant was used, displaying 3-fold higher burst strength than the original ApGltn (Org-ApGltn)/4S-PEG sealant, and 10-fold higher than the commercial fibrin sealant. Ste-ApGltn/4S-PEG-based sealant was biodegraded in rat subcutaneous tissue within 8 weeks without severe inflammation. By molecular interaction analysis using surface plasmon resonance, the binding constant of Ste-ApGltn to fibronectin was found to be 9-fold higher than that of Org-ApGltn. Therefore, the developed sealant, in particular the Ste-ApGltn/4S-PEG-based sealant, has potential applications in the field of cardiovascular surgery as well as thoracic surgery.

1. Introduction

Surgical sealants are widely used clinically for the treatment of pulmonary air leaks and anastomotic sites between living tissues. One commonly used sealant is fibrin sealant, and its components are biopolymers including fibrin and thrombin [1]. The sealing mechanism of this fibrin is based on human blood coagulation. Fibrin sealant has excellent biocompatibility and versatility; however, it does not possess sufficient sealing effect because of its low interfacial bonding strength to tissues. Therefore, the molecular design of a surgical sealant is required that will adhere to living tissue and organs under wet conditions during surgery.

Recently, a synthetic urethane-based sealant was developed for hemostasis of cardiovascular anastomosis [2]. It is composed of a viscous diisocyanate prepolymer, and forms urethane bonds between the isocyanate groups and amino groups in proteins. Urethane-based sealants have rapid curing characteristics and high bonding strength to soft tissues in the presence of water. In addition, they display load following capability because of their high elasticity. However, the polymerized product is non-absorbable and thus mediates adverse effects such as inflammation, infection and calcification [3].

Research into bio-inspired materials as surgical sealants has highlighted mussel-inspired adhesives as attractive candidates for sealing under wet conditions. It is known that the L-3, 4-dihydroxyphenylalanine in mussel adhesive proteins plays an important role in the adhesion of mussels in aquatic environments [4–7].

Nanomaterials have also been extensively studied as potential surgical sealants. Fujie et al. reported that a nanosheet composed of chitosan and alginate showed burst strength similar to fib-

* Corresponding author at: Polymeric Biomaterials Group, Biomaterials Field, Research Center for Functional Materials, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan.
E-mail address: taguchi.tetsushi@nims.go.jp (T. Taguchi).

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rin sealant, along with high biocompatibility and biodegradability [8–10]. Furthermore, adhesion using silica or iron oxide nanoparticles reveals nanobridging that achieves rapid and strong closure properties [11,12]. However, these nanomaterials have not yet demonstrated sufficient sealing effects for clinical applications.

In our previous study, we revealed that tissue adhesives containing hydrophobically-modified porcine-derived gelatins have excellent bonding strength onto fresh vascular media compared with non-modified gelatin [13–17]. However, high concentration porcine-derived gelatin solutions show low fluidity at room temperature because of their high content of imino acids, such as proline and hydroxyproline, and the need for heat treatment before sealant use.

Gelatin is one of the most popular biopolymers used in biomedical applications owing to its biodegradability and biocompatibility. Although porcine- and bovine-derived gelatin are most commonly used, in this study fish gelatin was employed owing to its unique properties [18]. The gelatin of cold-water fish in particular (*e.g.*, cod, hake, Alaska pollock, and salmon) shows higher fluidity compared with porcine- or bovine-derived gelatin [19].

Therefore, we have chosen Alaska-pollock-derived gelatins (ApGltn) instead of porcine-derived gelatin as a base material for surgical sealants [20], because it has a low transition temperature owing to its low content of imino acids [19,21,22]. We synthesized various hydrophobically-modified ApGltns (hm-ApGltn) with different hydrophobic groups and introduction ratios and evaluated their sealing effects on wet tissues by combining Hm-ApGltns with a poly(ethylene)glycol-based 4-armed crosslinker [23].

2. Materials and methods

2.1. Materials

Alaska-pollock-derived gelatin (ApGltn) was kindly donated by Nitta Gelatin Inc. (Osaka, Japan). Propanoyl chloride (Pro:C3), hexanoyl chloride (Hx:C6), lauroyl chloride (Lau:C12) and stearoyl chloride (Ste:C18) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (EtOH), ethyl acetate (EtOAc), dimethylsulfoxide (DMSO), triethylamine (TEA), 2,4,6-trinitrobenzensulfonic acid (TNBS), sodium dodecyl sulfate (SDS), 6 mol/L hydrochloric acid (6N-HCl), pyrene and acetone were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Pentaerythritol poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4S-PEG, Sunbright® PTE-100GS, MW = 10,000) was purchased from NOF Corporation (Tokyo, Japan). A porcine blood vessel was purchased from Funakoshi Corporation (Tokyo, Japan). Saline was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Collagenase was purchased from Nacalai Tesque Inc. (Kyoto, Japan).

2.2. Synthesis and characterization of hm-ApGltn

Modification of ApGltn with hydrophobic groups was carried out by a nucleophilic substitution reaction of the amino group of ApGltn with fatty acid chlorides (hydrocarbon chain length: C3–18) in DMSO (Fig. 1a) [13–17]. In brief, ApGltn (30 g) was first dissolved in DMSO (742.5 ml) at a concentration of 4 wt% at 37 °C. Fatty acid chlorides were then added into the ApGltn/DMSO solution at different concentrations from 10 to 40 mol% to change each theoretical introduction ratio. After stirring for 30 min, TEA (7.5 ml) was added to start the chemical reaction. The mixture was stirred for 15 h at room temperature in a N_2 atmosphere, hm-ApGltn was then precipitated in a mixture of cold ethanol and ethyl acetate. The precipitate was washed three times in cold ethanol (3 l) to remove any remaining fatty acid chlorides, DMSO and TEA. Hm-ApGltn was

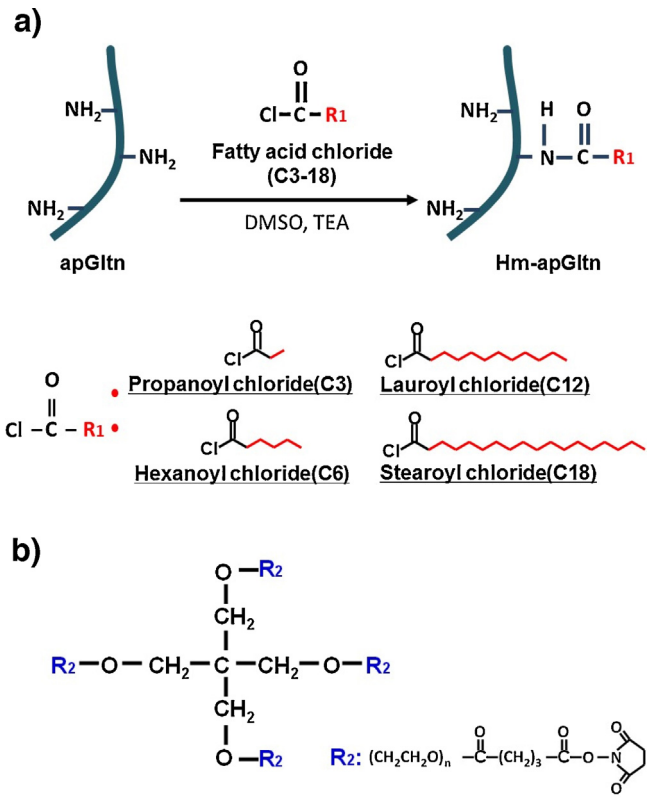

Fig. 1. Chemical structure of the components of sealants. (a) Preparation and structure of hm-ApGltn. (b) Chemical structure of pentaerythritol poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4S-PEG).

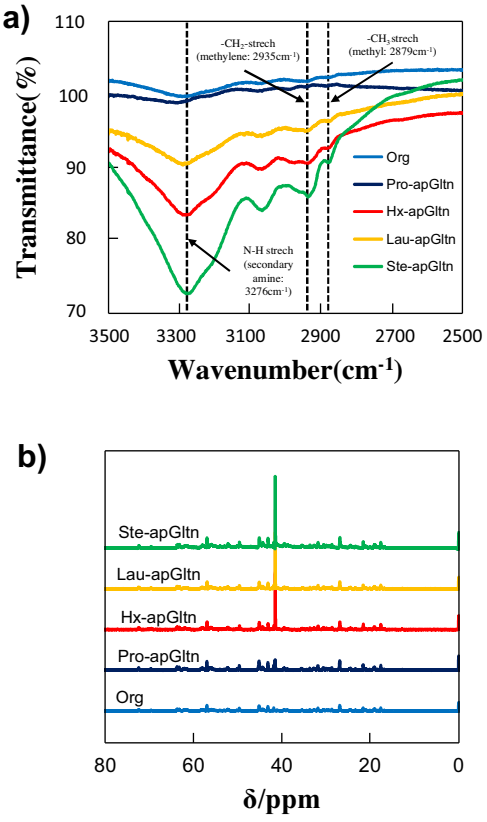

Fig. 2. Characterization of hm-ApGltn. (a) FTIR spectra of hm-ApGltn with different alkyl chain lengths. (b) ^{13}C NMR of hm-ApGltn with different alkyl chain lengths. The FTIR and ^{13}C NMR measurements were performed using Org-ApGltn, 6.4Pro-ApGltn, 8.5Hx-ApGltn, 9.0Lau-ApGltn and 9.6Ste-ApGltn.