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Enhanced biocompatibility and adhesive properties of modified allyl 2-cyanoacrylate-based elastic bio-glues

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ABSTRACT

Despite cyanoacrylate's numerous advantages such as good cosmetic results and fast application for first aid, drawbacks such as brittleness and local tissue toxicity have limited their applicability. In this study, to improve both the biocompatibility and mechanical properties of cyanoacrylate, allyl 2-cyanoacrylate (AC) was pre-polymerized and mixed with poly(L-lactide-co-ε-caprolactone) (PLCL, 50:50) as biodegradable elastomer. For various properties of pre-polymerized AC (PAC)/PLCL mixtures, bond strength, elasticity of flexure test as bending recovery, cell viability, and *in vivo* test using rat were conducted and enhanced mechanical properties and biocompatibility were confirmed. Especially, optimal condition for pre-polymerization of AC was determined to 150 °C for 40 min through cytotoxicity test. Bond strength of PAC/PLCL mixture was decreased (over 10 times) with increasing of PLCL. On the other hand, biocompatibility and flexibility were improved than commercial bio-glue. Optimal PAC/PLCL composition (4 g/20 mg) was determined through these tests. Furthermore, harmful side effects and infection were not observed by *in vivo* wound healing test. These results indicate that PAC/PLCL materials can be used widely as advanced bio-glues in various fields.

1. Introduction

Tissue adhesives have attracted growing interest as sealants, hemostatic agents, and non-invasive wound-closure materials. The adhesion of biological tissues is challenging in that the adhesive materials must satisfy several conditions, one of these is that suitable adhesion should be an attractive alternative to conventional sutures, rapid adhesion, and close apposition of wound edges for a sufficient period. Furthermore, such adhesive materials must not induce a marked inflammatory response, must be biodegradable, and must have minimal tissue toxicity [1].

Cyanoacrylates have been widely used as structural adhesives for metals, alloys, plastics, rubbers, and ceramics. Due to their biocompatibility and high reactivity under moist conditions, they are also used in medicine as surgical adhesives and coatings [2,3]. The main components of commercial medical cyanoacrylate adhesives for clinical applications are octyl 2-cyanoacrylate and butyl 2-cyanoacrylate, which are longer-chain derivatives [4,5]. However, the application of these polymers has been limited because of their unfavorable mechanical properties and the release of cytotoxic chemicals during their degradation [6,7]. Consequently, these materials are mainly used as biological adhesives in emergencies [2,8]. To overcome the limitations associated with these adhesives, researchers have investigated various cyanoacrylate modifications. Petrov reported that combining ethyl 2-cyanoacrylate and poly(methyl methacrylate) or poly(butadiene-co-acrylonitrile) improves the mechanical properties of the adhesive [9], while Tseng modified the cyanoacrylate monomer into ethoxyethyl cyanoacrylate by adding a side chain of relatively low hydrophobicity and high flexibility [10]. Allyl 2-cyanoacrylate (AC), an advanced cyanoacrylate derivative containing a double bond, has been introduced to enhance the mechanical properties of cyanoacrylate [7]. Although the mechanical properties of these polymers have been improved slightly by the abovementioned modifications, their limited biocompatibility for medical applications remains a challenge [11].

We previously reported that partial pre-polymerization of AC results in a longer chain structure, consequently improving the biocompatibility and stability of cyanoacrylate [7]. The application of this product to areas of body movement such as joints, however, remained limited due to the poor flexibility of the product. Thus, there remains a need for similar products with improved

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mechanical properties including bond strength and flexibility as well as enhanced biocompatibility of tissue adhesives for medical applications.

In this study, the biodegradable elastomer poly(L-lactide-co-ε-caprolactone) (PLCL) was chosen as an additive for elastic pre-polymerized AC (PAC). This copolymer was chosen based on the different elastic recoveries of amorphous polymers and highly crystalline polymers depending on the lactide/caprolactone molar ratio of the material, as previously reported [12]. PLCL is usually used in studies on transplanted biomaterials such as tissue engineered blood vessels, cartilage, and skin [13,14].

The objectives of this study were (1) to select the conditions that yielded the most stable blend of PLCL and PAC for use as elastic bio-glue, (2) to determine the PLCL and PAC compositions that yielded the optimal mechanical parameters, and (3) to evaluate the cytotoxicity of the selected bio-glue. This study is thus an *in vitro* and *in vivo* assessment of the biocompatibility of the optimum PAC/PLCL mixture for clinical application as a tissue adhesive.

2. Materials and methods

2.1. Preparation and characterization of PACs

AC (920; Robinson St. Pottstown, PA, USA) was heated at 150 °C for 0, 5, 10, 20, 40, and 60 min in vacuum vials (10 ml/vial). Samples were then cooled to 0 °C and stored at 4 °C. Change of viscosity according to heating time was measured using viscometer (Brookfield, model LVDV-II+P, Middleboro, MA, USA) and compared with ethyl 2-cyanoacrylate as control.

2.2. Cytotoxicity test of PACs

As a direct contact method, the cytotoxicity of PAC was tested using a previously reported method [15]. L929 cells (ATCC, Manassas, VA, USA) were cultured in RPMI 1640D media with 20% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin. The cells were added in 24 well plate (10^4 cells/well) and maintained in a humidified atmosphere that contained 5% CO_2 at 37 °C. After 24 h, the media was aspirated and PACs and Dermabond of 100 μl were each added directly to the center of each cultured well. After another 1 h, 2 ml media were slowly added to each well. After 4 h and 24 h, the initial adhesion and proliferation were determined by using a MTT assay. To determine viability, absorbance was measured at a test wavelength of 570 nm using a microplate reader (Molecular Devices, Toronto, Canada). The optical density (O.D.) was calculated as the difference between the reference wavelength and the test wavelength. The percent viability was calculated as [(O.D. of drug-treated sample/O.D. of untreated sample) × 100].

2.3. Preparation of the PAC/PLCL mixtures

PLCL [molar ratio 50:50, number-average molecular weight (Mn): 2.2×10^5] was polymerized and purified using a method described previously [10]. The 2% (w/v) PLCL was dissolved in chloroform solution. PLCL solution of various volumes was mixed with a fixed volume of PAC, as presented in Table 1. The mixtures were shaken for 1 h at room temperature and dried in a heat-drying oven for 7 h at 65 °C to completely remove the chloroform solvent.

Table 1
Composition of pre-polymerized ally 2-cyanoacrylate (PAC)/poly(L-lactide-co-ε-caprolactone) (PLCL) mixtures.

Mixture	PAC (g)	PLCL (mg)
Type-1	4	20
Type-2	4	40
Type-3	4	80
Type-4	4	120

2.4. Elasticity of flexure test of the PAC/PLCL mixtures

Films (size = 10 mm × 50 mm × 0.03 mm) with various PAC/PLCL ratios were prepared by natural drying in 10 wt.% chloroform solutions for 3 weeks. The elasticity of flexure test was performed using a universal testing machine (Instron model 5966, Canton, MAUSA). A 5 N load cell with a crosshead speed of 1 mm/min was used for this experiment. Each film was folded in half at a folding speed of 1 mm/min, immediately after which the stress was released and the recovered angle for 5 min was measured and compared with that of an octyl 2-cyanoacrylate film. The percent elasticity of flexure was calculated as [(recovered angle of film/angle (180°) of unfolded film) × 100].

2.5. Bond strengths of the PAC/PLCL mixtures

The PAC/PLCL mixtures were applied to bovine skin (area: 10 mm × 10 mm). The specimens were then covered with another section of bovine skin. The volume of sample was 10 μl, and the final adhesion thickness was 10 μm. After 24 h at room temperature, the bond-strength of the PAC/PLCL mixtures was tested using a universal testing machine (Instron model 4467). The crosshead speed was set to 1 mm/min, and the load at which the specimen deboned from the adherent was recorded.

2.6. In vitro *cytotoxicity test of the optimum PAC/PLCL mixture*

Cytotoxicity testing of the optimum PAC/PLCL mixture was performed using direct contact method (Section 2.2).

2.7. Epidermal growth factor (EGF) release test

An EGF (Sigma–Aldrich, St. Louis, MO, USA) solution (500 μg/ml) and chitosan (>1000 kDa, 86% DD, Korea Chitosan Co., Ltd.) of 1 wt.% in 1% acetic acid solution was prepared and dropped in liquid nitrogen using a syringe pump (rate: 1 mm/min). And then, the beads were lyophilized for 24 h. The Bradford protein assay was used to measure the EGF released from the chitosan micro-beads, prepared by the EGF included chitosan micro-beads (dry weight 10 mg) and EGF included chitosan micro-beads (dry weight 10 mg)/PAC/PLCL (1 g) mixture, respectively. The samples were suspended in 10 ml PBS and incubated at 37 °C and 30 rpm for 0, 2, 5, 8, 15, 20, 45, and 60 min. After the incubation, the releasate, condensed by lyophilization, was added to a 96-well plate. The Bradford reagent (Bio-Rad) was then added to each well before incubating the plate in the dark at room temperature for 15 min. Absorbance was measured at 595 nm after shaking for 30 s.

2.8. In vivo *study*

Fifteen young male Sprague–Dawley rats (weight, 250–350 g) were used. All animals were anesthetized with inhaled isoflurane (1–5%). After anesthesia, hair from the animal's back was removed with electric clippers, and a depilatory cream (Nair; Carter Products) was applied for several minutes. The area was washed with wet cotton and dried with a gauze sponge. Povidone-iodine was applied to the surgical site and removed, and then isopropyl alcohol (70%) was applied. Two 1.5 cm long incisions were made on the back of each rat, with at least 1 cm of intact skin between them. The EGF included chitosan micro-beads (20 mg) were applied to