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Enhanced biocompatibility and wound healing properties of biodegradable polymer-modified allyl 2-cyanoacrylate tissue adhesive

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ABSTRACT

As poly L-lactic acid (PLLA) is a polymer with good biocompatibility and biodegradability, we created a new tissue adhesive (TA), pre-polymerized allyl 2-cyanoacrylate (PACA) mixed with PLLA in an effort to improve biocompatibility and mechanical properties in healing dermal wound tissue. We determined optimal mixing ratios of PACA and PLLA based on their bond strengths and chemical structures analyzed by the thermal gravimetric analysis (TGA) and Fourier transform infrared (FT-IR) spectroscopy. In vitro biocompatibility of the PACA/PLLA was evaluated using direct- and indirect-contact methods according to the ISO-10993 cytotoxicity test for medical devices. The PACA/PLLA have similar or even better biocompatibility than those of commercially available cyanoacrylate (CA)-based TAs such as Dermabond® and Histoacryl®. The PACA/PLLA were not different from those exposed to Dermabond® and Histoacryl® in Raman spectra when biochemical changes of protein and DNA/RNA underlying during cell death were compared utilizing Raman spectroscopy. Histological analysis revealed that incised dermal tissues of rats treated with PACA/PLLA showed less inflammatory signs and enhanced collagen formation compared to those treated with Dermabond® or Histoacryl®. Of note, tissues treated with PACA/PLLA were stronger in the tensile strength compared to those treated with the commercially available TAs. Therefore, taking all the results into consideration, the PACA/PLLA we created might be a clinically useful TA for treating dermal wounds.

1. Introduction

Tissue adhesives (TAs) have been considered as a wound closure/sealant for surgical and hemostatic procedures [1]. Ideal properties of surgical TA include good binding strength, simplicity of application, biocompatibility, biodegradability, minimal tissue toxicity and cost-effectiveness [2]. TAs can be divided into synthetic and biological types. Synthetic TAs include cyanoacrylate (CA)- and natural polymer-based adhesives, and biological TAs include proteins such as fibrin and gelatin. In general, synthetic TAs are less expensive and easier to produce compared to biological TAs [3]. In particular, CA-based synthetic TAs are most commonly used for surgical procedures. The potential benefits of usage of CA-based synthetic TAs include effective wound closure process, speedy operation, better cosmetic outcomes, reduced discomfort and risk of infection [4–6]. Nonetheless, CA-based synthetic TAs also have limitations such as mechanical rigidness and potentially hazardous biodegraded byproducts. Therefore, considerable amounts of efforts have been made to develop alternative synthetic TAs with improved biocompatibility and mechanical strength.

Toxicity of the CA-based synthetic TAs can be reduced by the longer length of their side chain [7–10], and as a result of much research efforts, alternative TAs such as octyl-2-CA and n-butyl-CA with a longer side chain have been developed and marketed with commercial brand names, Dermabond® (Johnson & Johnson/Ethicon, Somerville, NJ) and Histoacryl® (B. Braun, Melsungen, Germany), respectively [8–11]. Recently, pre-polymerization process has been demonstrated to allow allyl 2-CA (PACA) to result in a longer chain structure and better for biocompatibility when tested both in vitro and in vivo [12]. Additionally, various natural polymer-based synthetic TAs have been discovered as hydrophilic, biocompatible and non-toxic materials. For example, chitosan, a prominent wound closure material candidate for natural polymer-based synthetic TA has many advantages with its tissue compatibility, hemostatic activity, low toxicity, biodegradability and anti-infection activity [13,14]. It also has limitations with its structural rigidity and poor solubility in water [15,16]. Although chitosan-based TAs can be modified to become stronger than fibrin-based TAs in bonding strength, they are not as strong as CA-based TAs [17,18]. Poly L-lactic acid (PLLA) is one of the most promising new materials for tissue repair applications which has been approved by the United States Food and Drug Administration with good mechanical strength, biodegradability

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and biocompatibility [19–24]. The quality of PLLA as TA has been improved for rheological and adhesive properties by optimizing oligomer chain length and composition [25,26].

In this study, we attempted to further improve the biocompatibility and mechanical strength of PACA by mixing with PLLA. To this end, we investigated the biocompatibility and wound healing efficiency of the mixtures of PACA and PLLA (PACA/PLLA) and compared their qualities with those of commercially available Dermabond® and Histoacryl®.

2. Material and methods

2.1. PACA/PLLA preparation and characterization

2.1.1. PACA/PLLA preparation

To prepare PACA, ACA (ACA 920, Permabond Engineering Adhesives, Pottstown, PA, USA) was heated at 150 °C for 40 min in vacuum vials (10 ml/vial) and then cooled to 0 °C. The viscosity measured with viscometer (LVDV-II + P, Brookfield Engineering, Middleboro, MA USA) was compared with that of 2-ethyl cyanoacrylate (ECA) used as a control. PLLA with Mw ~ 260,000 and impurities ≤ 0.5% water was purchased from Sigma Aldrich, USA. PLLA was dissolved in chloroform at the concentration of 10% (w/v). 10% (w/v) PLLA solution of various volumes (0.5, 3 and 6 ml) was mixed with a fixed volume (4 ml) of PACA, as presented in Table 1. The mixtures were shaken for 1 h at room temperature (RT) and then dried in a heat-drying oven for 5–7 h at 65 °C to completely remove the chloroform solvent. The mixtures were then kept in a sterile plastic container under aseptic conditions. Thermal gravimetric analysis (TGA), Fourier transform infrared (FT-IR) spectroscopy, bond strength tests, and cell viability tests were carried out to characterize the physiochemical properties of PACA/PLLA mixtures.

2.1.2. PACA/PLLA thermal and structural properties

TGA (Netzsch 209 F1, Waltham, MA, USA) was performed to determine the PACA/PLLA mixtures, as assessed by weight loss of the materials that resulted from increasing temperature. PACA/PLLA mixtures (10–15 mg) were heated from RT up to 500 °C at a heat acceleration rate of 3 °C/min in flowing argon (99.9999%, 40 ml/min). IR spectra were obtained from a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany) configured in a wavenumber range of 4000–400 cm^{-1} and spectral resolution of 4 cm^{-1} using a DLATGS detector and a KBr beam splitter.

2.1.3. PACA/PLLA bond strengths

Bond strengths of PACA/PLLA mixtures were assessed according to Adhesive standard test methods (ASTM) F2255-05. Briefly, PACA/PLLA mixtures at various ratios were applied to bovine skin (bonding area: 10 × 10 mm^2). 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 curing for 24 h at room temperature, bond strength of the PACA/PLLA mixtures was tested using a universal testing machine (Instron model 4467). The crosshead speed was set to 5 mm/min, and the load at which the specimen deboned from the adhesive was recorded (n = 3).

Table 1
Mixed composition of pre-polymerized ally 2-cyanoacrylate (PACA) and poly L-lactic acid (PLLA).

	Prepolymer of allyl 2-CA (PACA, ml)	Poly L-lactic acid (10% (w/v) PLLA, ml)
PACA/PLLA0.0125	4	0.5
PACA/PLLA0.075	4	3.0
PACA/PLLA0.15	4	6.0

2.2. Cell culture

The Mouse L929 fibroblast cells were obtained from the Korea Cell Line Bank (NCTC clone 929, Seoul, South Korea). Cells were grown in RPMI 1640 (GIBCO, Grand Island, NY, USA) supplemented with 300 mg/ml L-glutamate, 25 mM hydroxyethyl piperazineethanesulfonic acid (HEPES) buffer, 25 mM $NaHCO_3$, 10% fetal bovine serum (FBS), 50 μg/μl gentamicin, 500 U/ml penicillin and 500 mg/ml streptomycin in a 5% CO_2 incubator at 37 °C under a humidified atmosphere.

2.3. Cell viability tests

2.3.1. Water-soluble tetrazolium salt (WST) assay by direct contact method

L929 fibroblasts (1×10^5 cells/1 ml, 70–80% cell density) were seeded in 12-well plates and incubated for 24 h. First, we determined the half maximal inhibitory concentration (IC_{50}) value for the Dermabond® as 5 μl/10^5 cells. Same volume (5 μl) of PACA/PLLA, Dermabond® and Histoacryl® was placed at the edge of the 12-well plates and incubated at 37 °C in a 5% CO_2 incubator. After 24 h, cell viability was measured using a cell counting kit-8 (Sigma Aldrich, St. Louis, MO, USA). The OD_{450} was recorded by a multi-microplate reader (Synergy HT multimode microplate instrument, BioTek, Winooski, VT, USA). All experiments were performed in triplicate. The results were averaged and expressed as percentages of the controls without TAs.

In addition, to examine the time course of cell viability, cells were treated with PACA/PLLA, Dermabond® and Histoacryl® and then incubated for 24, 48 and 72 h. Cell viability was assessed using the WST assay.

2.3.2. LIVE/DEAD assay by direct contact method

Approximately 1×10^5 L929 cells (70% cell density) were seeded in a 35-mm glass bottom dish and incubated for 24 h. After the exposure of TAs for 24 h, cell viability was measured by a LIVE/DEAD viability/cytotoxicity kit (Molecular Probes, Eugene, OR, USA). Positive (70% methyl alcohol) and negative (untreated TA) controls were prepared to verify the test system. The images of the labeled cells were obtained using a Zeiss LSM-700 confocal microscopy system (Thornwood, NY, USA). The images are one representative of the results from three independent experiments. The numbers of viable (green) and non-viable (red) cells were counted automatically with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.3.3. Agar overlay assay by indirect contact method

Cells (5×10^5 cells, 70–80% cell density) were seeded in 100-mm dishes. The cells were incubated for 24 h at 37 °C, to allow them to adhere to culture dish as a monolayer confluence. The medium was removed and replaced with 1.5% agar containing 0.01% Neutral Red (NR), and then incubated for additional 20 min. Excessive dye was removed from cells and three TAs, negative (growth medium) and positive (70% methyl alcohol) controls were applied onto the agar surface. Following additional 48 h of incubation at 37 °C in a 5% CO_2 incubator, the dishes were examined under an inverted microscope (Nikon Eclipse Ti, Nikon, Tokyo, Japan). The degree of decolorized zones and cell lysis around the specimens was evaluated according to ISO-10993-5. Cytotoxic biological relativities were rated on a scale from Grade 0 (No reactivity) to Grade 4 (Severe reactivity). Grade 2 (mild) or less is considered to be non-cytotoxic. All the experiments were performed four times.

2.3.4. Elution assay by indirect contact method

IC_{50} for Dermabond® is 5 μl/10^5 cells. The same volume (5 μl) of PACA/PLLA, Dermabond® and Histoacryl® was added in RPMI medium (1 ml), and then collected elution mediums of three TAs at various times including 0 h, 3 h, 6 h, 12 h, 24 h, 3 d, 5 d, 7 d, 3 wk and 5 wk. Zero (0 h) time samples were not exposed to TA. Cells (1×10^5) were seeded and incubated for 24 h. Cells were treated with each elution medium