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Enhanced biocompatibility and adhesive properties by aromatic amino acid-modified allyl 2-cyanoacrylate-based bio-glue

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ABSTRACT

Cyanoacrylates have numerous advantages, including that they can be applied quickly during first aid and can provide good cosmetic outcomes, but they also have limitations in that they have a low bond strength and local tissue toxicity. Consequently, they are primarily used only in urgent applications. To improve both the biocompatibility and the mechanical properties of cyanoacrylate, allyl 2-cyanoacrylate (AC) was prepolymerized and mixed with a dopamine co-initiator. Various properties of prepolymerized AC (PAC)/dopamine mixtures were tested using mouse fibroblast cell (L-929), including their bond strength, setting time, crystallization intensity, and cytotoxicity. Enhanced mechanical properties and biocompatibility were confirmed, and a cytotoxicity test was used to determine the optimal conditions for prepolymerization of AC to be 130 °C for 60 min. A combination of 5 mg of dopamine in 5 ml of PAC achieved a high bond strength with cytotoxicity of the dopamine/PAC at approximately 1.5 times lower than that of PAC. These results indicate that dopamine/PAC materials can be extensively used as advanced bio-glues in various applications.

1. Introduction

Cyanoacrylates have recently gained wide acceptance for use as structural adhesives for metals, plastics, rubbers, and ceramics [1,2]. In addition, they are also used as surgical adhesives and coatings in medicine due to their bacteriostatic [3] and hemostatic properties [4–6] and their high reactivity in moist conditions [7].

Despite these advantages, the low bond strength and local tissue toxicity limit the applicability of cyanoacrylates, and consequently, they are only used as adhesives in situations that demand urgent application [7–10].

To overcome these limitations, researchers have attempted to implement various modified versions of cyanoacrylates. Examples of this include mixing other polymers with cyanoacrylates, and then adding acetyl tri-n-butyl citrate as a plasticizer and modifying the intra-cyanoacrylate alkyl group [7–12]. However, such modifications yielded only limited control with respect to the mechanical properties of the polymer, and the cytotoxicity remains unresolved, rendering the materials unsuitable for medical applications [12,13].

Recently, prepolymerized allyl 2-cyanoacrylate (PAC), an advanced cyanoacrylate formed with partially polymerized intramolecular double bonds, has been introduced as a bio-glue. Partial prepolymerization of allyl 2-cyanoacrylate (AC) has been reported to improve biocompatibility and stability due to having a longer chain structure [14,15]. However, many researchers have observed a reduced bond strength that results in having longer chains [8,14,16].

Therefore, use of additives that improve the adhesion and mechanical properties of the materials is necessary in order to enhance the properties of the cyanoacrylates as bio-glues. When cyanoacrylates are exposed to anions, such as the hydroxyl moiety found in water or the various anions found in blood and various nucleophiles, polymerization is initiated, and the ethylene units bind together within a few seconds at room temperature. This process induces the formation of strong adhesive bonds [17]. The amines are sufficiently nucleophilic to initiate cyanoacrylate polymerization, and the reaction mechanism involves a Michael-type addition of initiator across the cyanoacrylate double bond to produce a zwitterion that subsequently reacts with an additional monomer to finally form the adhesive polymer [18,19].

In this study, L-3,4-dihydroxyphenylalanine (L-DOPA) and dopamine were used as biocompatible aromatic amino acid initiators in order to enhance the adhesive bond strength and biocompatibility of the cyanoacrylates.

The objectives of this study were as follows: (1) to determine the optimal initiator among L-DOPA and dopamine, (2) to evaluate the

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Table 1
Composition of bio-glues.

Samples	[a]L-DOPA (mg)	Dopamine (mg)	[b]PAC (ml)
L-DOPA-0 or PAC	0	0	5
L-DOPA-1	1	0	5
L-DOPA-5	5	0	5
L-DOPA-10	10	0	5
Dopamine-0 or PAC	0	0	5
Dopamine-1	0	1	5
Dopamine-5	0	5	5
Dopamine-10	0	10	5

[a] L-3,4-dihydroxyphenylalanine.
[b] Prepolymerized allyl 2-cyanoacrylate.

influence of the aromatic amino acid treatment on the cytotoxicity of the materials, and (3) to determine the optimal composition of PAC/aromatic amino acids to improve the adhesive properties.

2. Materials and methods

2.1. Preparation and characterization of PACs

1 N HCl solution with AC (920; Robinson St. Pottstown, PA, USA) was heated at 130 °C for 0, 10, 20, 30, 40, 50, 60, and 70 min in vacuum vials (1 N HCl of 0.1 ml with AC of 9.9 ml/vial). Samples were separated from the HCl solution with added *n*-hexane. The *n*-hexane was removed using an evaporator, and then the remaining PACs were cooled to 0 °C and were stored at 4 °C. The change in viscosity with respect to the heating time was measured using a viscometer (Brookfield, model LVDV-II + P, Middleboro, USA).

2.2. Cytotoxicity test of PACs

In this study, a cytotoxicity test with a direct contact method was conducted according to ISO 10993 (Biological evaluation of medical devices). L929 cells (ATCC, Manassas, VA, USA) were cultured in RPMI 1640D media supplemented with 20% fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin. The cells were added to a 24-well plate (10^4 cells/well) and were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. After 24 h, the media were aspirated, and the PACs that were heated to 0, 10, 20, 30, 40, 50, or 60 min were added directly to the center of each cultured well along with octyl 2-cyanoacrylate as the main component of 100 μl Dermabond. After another 1 h, 2 ml of media were slowly added to each well, and after 72 h, cell proliferation of each of the PACs of different heating times was determined using an MTT assay [20]. To determine cell 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. Finally, the percent viability was calculated as [(O.D. of bio-glue-treated sample/O.D. of untreated sample) × 100].

2.3. Preparation of the L-DOPA or dopamine/PAC mixtures

Dopamine and L-DOPA at various concentrations were mixed with PAC. The mixture was then stirred at room temperature, as listed in Table 1.

2.4. Setting time of L-DOPA or dopamine/PAC mixtures

To determine the setting time of the dopamine/PAC and L-DOPA/PAC mixtures, the ingredients were first mixed according to the compositions given in Table 1. The viscosity change at 23 °C with a small oscillation mode (1 Hz) was measured using a rheometer (CVO 100, Bohlin Instruments, Worcestershire, UK) that was equipped with a 1° cone and 40-mm plate tools. The setting time was defined as the point at which a sharp transition was observed in the viscous modulus (G'').

2.5. In vitro cytotoxicity test of L-DOPA or dopamine/PAC mixtures

A direct contact method (Section 2.2) was used to test the cytotoxicity of the PAC with L-DOPA or dopamine mixtures (0, 1, 5, and 10 mg in 5 ml of PAC; Table 2).

2.6. Crystal intensity of dopamine/PAC mixture

The crystal intensity diagrams of the dopamine/PAC bio-glues were measured via X-ray diffraction (XRD: D8 advance; Bruker AXS, Karlsruhe, Germany) operating with Cu-Kα radiation (λ = 0.15406 nm) at 40 kV with current of 100 mA at a speed of 1°/min.

2.7. Thermal stability of the dopamine/PAC mixture

Thermo-gravimetric analysis (TGA) was conducted on a Hi-Res TGA 2950 (TA Instrument) under N_2 flow. Samples were heated to 300 °C at a heating rate of 5 °C/min.

2.8. Bond strengths of dopamine/PAC mixtures

A piece of cowhide with a size of 50 × 10 × 1.5 mm^3 was used to test the bond strength. Control, octyl 2-cyanoacrylate as a commercial bio-glue for soft tissue, and dopamine/PAC mixtures of each 100 μl were applied to one end of the cowhide in an area of 10 × 10 mm^2, and the specimens were covered by another section of cowhide. After 24 h at room temperature, a bond strength test was performed using a universal testing machine (Instron model 4467, Canton, MA, USA). The crosshead speed was set to 1 mm/min, and the maximum load was recorded before the layers of skin were completely separated.

2.9. Confocal laser scanning microscopy for surface analysis of adhesion area on the cowhide

For the permeability test on the cowhide of the dopamine/PAC mixture with PAC, 500 μl polymethacrylic acid (PMA; Mw: 9,500, Sigma–Aldrich) was added to cowhide (10 × 10 mm^2) under 0.1 MPa vacuum conditions for 48 h, and dried PMA film was carefully removed from the cowhide. 100 μl of glue were added on the adhesion area of the removed PMA film from the cowhide. After 24 h at room temperature, the PMA film was dissolved in distilled water for 5 h, and the surface roughness on the adhesion area of the remaining polymerized glue was observed using a confocal laser scanning microscope (Carl Zeiss Olympus-FV300; Tokyo, Japan).

3. Results and discussion

3.1. Characterization of prepolymerized allyl 2-cyanoacrylates

Various tests, including viscosity and cytotoxicity tests, were conducted to find the optimal prepolymerization conditions, and the results were compared to those of octyl 2-cyanoacrylate. The yellowish color and viscosity of the samples increased as the heating time increased, particularly in the PAC samples. We suspect that the increased viscosity of the PAC could be attributed to a partial polymerization of another intramolecular double bond and not to a polymerizable double bond of an anion initiator. Fig. 1 shows