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A mussel-inspired double-crosslinked tissue adhesive intended for internal medical use

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ABSTRACT

It has been a great challenge to develop aldehyde-free tissue adhesives that can function rapidly and controllably on wet internal tissues with fine adhesion strength, sound biocompatibility and degradability. To this end, we have devised a mussel-inspired easy-to-use double-crosslink tissue adhesive (DCTA) comprising a dopamine-conjugated gelatin macromer, a rapid crosslinker (namely, Fe^{3+}), and a long-term acting crosslinker (namely, genipin). As a mussel-inspired gluing macromer, dopamine is grafted onto gelatin backbone via an one-step reaction, the catechol groups of which are capable of performing strong wet adhesion on tissue surfaces. By addition of genipin and Fe^{3+}, the formation of catechol–Fe^{3+} complexation and accompanying spontaneous curing of genipin-primed covalent crosslinking of gluing macromers in one pot endows DCTA with the double-crosslink adhesion mechanism. Namely, the reversible catechol–Fe^{3+} crosslinking executes an controllable and instant adhesive curing; while genipin-induced stable covalent crosslinking promises it with long-term effectiveness. This novel DCTA exhibits significantly higher wet tissue adhesion capability than the commercially available fibrin glue when applied on wet porcine skin and cartilage. In addition, this DCTA also demonstrates fine elasticity, sound biodegradability, and biocompatibility when contacting in vitro cultured cells and blood. In vivo biocompatibility and biodegradability are checked and confirmed via trials of subcutaneous implantation in nude mice model. This newly developed DCTA may be a highly promising product as a biological glue for internal medical use including internal tissue adhesion, sealing, and hemostasis.

Statement of Significance

There is a great demand for ideal tissue adhesives that can be widely used in gluing wet internal tissues. Here, we have devised a mussel-inspired easy-to-use double-crosslink tissue adhesive (DCTA) that meets the conditions as an ideal tissue adhesive. It is composed of gelatin–dopamine conjugates – a gluing macromer, Fe^{3+} – a rapid crosslinker, and genipin – a long-term acting crosslinker. This DCTA is constructed with a novel complexation-covalent double-crosslinking principle in one pot, in which the catechol–Fe^{3+} crosslinking executes a controllable and instant adhesive curing, at the same time, genipin-induced covalent crosslinking promises it with long-term effectiveness in physiology conditions. This novel DCTA, with excellent wet tissue adhesion capability, fine elasticity, sound biodegradability, and biocompatibility, is a promising biological glue for internal medical use in surgical operations.

1. Introduction

Medical adhesives have facilitated surgical operations for several decades, particularly in the cases when traditional suturing poses impractical or ineffective [1,2]. Fibrin glues (such as Tisseel) [3], albumin-glutaraldehyde adhesives (such as BioGlue) [4], and cyanoacrylates (such as Dermabond) [5] are well-known and currently used in many surgical procedures. However, the utilization of fibrin glues involves risks of blood-borne disease transmission and allergic reactions to patients [6]; the high toxicities of aldehyde-containing products severely limit in vivo applications of the revelant adhesive products [7,8]. Currently, there is few commercially available tissue adhesive that can be

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widely used for in vivo applications such as internal tissue or organ adhesion and hemostasis [9].

Mussel adhesive proteins (MAPs) secreted by marine mussels are known capable of building complexes of byssal threads and adhesive plaques to anchor their own bodies firmly onto all surfaces [10]. This finding fascinates the researchers particularly due to their biocompatibility and unique wet adhesion capability [11–14]. Studies have revealed that the excellent affinity of MAPs to wet surfaces can be primarily attributed to the unique catecholic amino acid, L-3,4-dihydroxyphenylalanine (DOPA) [13,15,16]; the pendant catechol group of which is directly responsible for the moisture-resistant adhesion [13,17]. The adhesion mechanism consists of two simultaneous procedures: first, the catechol moieties generate strong non-covalent binding interactions to various surfaces [13,17,23]; second, as a catechol-containing adhesive biopolymer, MAPs are crosslinked by various means, among which the natural way is to convert catechol groups into quinones by polyphenol oxidase enzyme in mussels and then achieve covalent self-crosslinking [13,16,23]. On top of the self-polymerization, strong and reversible coordination interactions between catechol groups and metal ions from ambient environment, such as Fe^{3+}, Cu^{2+}, Ti^{3+}, are widely considered to further strengthen the crosslink to achieve the final hardness [23,45]. Particularly, the catechol–Fe^{3+} coordination complexation can be rapidly established, which is reversible to serve as a sacrificial load-bearing crosslink to facilitate the extensibility of byssus and play a crucial role in mussels' adhesion to wet surfaces in turbulent environments [24,26,27,45].

In order to apply these mechanisms to adhesion of biological tissues or organs, various mussel-inspired adhesive (bio)polymers have been developed by incorporating and adopting typical catechol-containing molecule, like dopamine [19,20] or hydrocaffeic acid [21,22], as the functional component for wet-resistant tissue adhesives. Chung et al. have synthesized DOPA-containing adhesive terpolymers that can be cross-linked through the reaction between N-hydroxysuccinimide ester and thiol group without sacrificing DOPA moieties. The adhesion tests of the resultant adhesives indicate the catechol moieties provide a driving force for its adhesion to wet porcine skin [25]. Further studies particularly on crosslinking procedure have shown the catechol group can be oxidized under oxidative or alkaline conditions to produce quinones [9,13]. Several polymeric tissue adhesives are correspondingly developed, and the catechol groups of which are oxidized into quinones by using oxidizing reagents (e.g. sodium periodate, hydrogen peroxide) [9,20,21]. By this means, not only does the quinone trigger intermolecular cross-linking of adhesive polymers but also leads to strong adhesion to tissues through reaction with available nucleophile groups (e.g. $-NH_2$, $-SH$) on tissues' surfaces [9,13,20,21]. In addition, the catechol–Fe^{3+} coordination complexation is also exploited [18,24,26–29]. Previously, it has been adopted to fabricate self-healing hydrogels due to the reversibility [28]. Choi et al. develop a hemostatic hydrogel based on rapidly achieved catechol–Fe^{3+} coordination crosslinking [29]. The intriguing characteristics of catechol–Fe^{3+} crosslinking, namely the combination of rapid establishment and reversibility, make it a promising strategy for fabricating tissue adhesives.

In this study, we have developed a mussel-inspired synthetic double-crosslink tissue adhesive (DCTA) comprising a dopamine-conjugated gelatin macromer, a rapid crosslinker (namely, Fe^{3+}), and a long-term acting crosslinker (namely, genipin). As a mussel-inspired gluing macromer, the dopamine served as interfacial adhesion segment, is grafted onto gelatin backbone via EDC/NHS coupling chemistry. By addition of Fe^{3+}, rapid formation of catechol–Fe^{3+} complexation endows the first crosslinking mechanism of gluing. Albeit instant as it is, the reversibility of this complexation reaction makes it unstable afterwards. A second crosslinker, genipin, is applied at the same time. Genipin is a natural product and nontoxic crosslinker that can spontaneously react with the primary amino groups in polymers (such as gelatin, chitosan, polylysine) to form distinctive blue pigments [30–33]. Genipin has been considered as a promising crosslinker to replace glutaraldehyde for the preparation of biomaterials due to its advantage of biocompatibility [31]. However, the genipin-based crosslinking process needs to cost much time (tens of minutes to hours) that is unacceptable to cure adhesive polymers under clinical conditions [32,33]. Single usage of either Fe^{3+} or genipin as crosslinker of tissue adhesive cannot meet the demand for the rapid cross-linking and long-term effectiveness in clinical conditions. This novel DCTA, mimicking the double-crosslinking of MAPs in the byssal cuticle [9,23], is fabricated in one pot via a double-crosslink mechanism. Namely, catechol–Fe^{3+} complexation executes an instant adhesive curing; while genipin-primed covalent crosslinking promises it with long-term effectiveness under physiological conditions. The formation, adhesion strength, physical properties, degradation profile as well as in vitro cytocompatibility and blood compatibility of DCTA are evaluated in detail. The subcutaneous implantation of DCTA is also carried out to further assess its biocompatibility and degradation under in vivo conditions.

2. Materials and methods

2.1. Synthesis of gelatin–dopamine gluing macromer

Gelatin–dopamine conjugate is synthesized as a typical gluing macromer through ethyl-dimethyl-aminopropylcarbodiimide (EDC, Sigma–Aldrich) and N–hydroxy-succinimide (NHS, Sigma–Aldrich) coupling chemistry. Briefly, gelatin (2.0 g, Type A from porcine skin, Sigma–Aldrich) is dissolved in 100 mL of phosphate buffered saline (PBS, pH 7.4) solution at 60 °C. EDC (0.5 g) and NHS (0.3 g) is added into the solution and pH value of the mix solution is adjusted to ~5.0. After 30 minutes' stirring, 1.0 g of dopamine hydrochloride (Sigma–Aldrich) dissolved in 2 mL of deionized (DI) water is added dropwise and pH value of the reaction solution is maintained from 5.0 to 6.0 for 24 h at 37 °C. Subsequently, the reaction solution is dialyzed in DI water for two days and then lyophilized.

2.2. Characterization of gelatin–dopamine gluing macromer

To confirm the successful grafting of dopamine onto gelatin, the resultant gelatin–dopamine conjugate is analyzed by proton nuclear resonance spectroscopy (^{1}H NMR). 1.5% (g/mL) of gelatin–dopamine solution in deuterium oxide (D_2O) is transferred into a 5 mm NMR tube and recorded on a Bruker Avance-300 NMR spectrometer. Besides, the ^{1}H NMR spectra of parent materials, namely gelatin and dopamine, are also collected.

Furthermore, the presence of unoxidzied catechol groups in gelatin–dopamine conjugate is assessed by UV–Vis spectroscopy [9,29]. 10% (g/mL) of gelatin and gelatin–dopamine solutions in DI water are scanned, respectively, at wavelengths from 250 nm to 500 nm using a Nanodrop 2000c spectrophotometer (Thermo Scientific).

The content of catechol group is determined with Arnow's method [34], in which dopamine is used as standard. Typically, 1.0 mL of nitrite-molybdate reagent, namely 10% (g/mL) of sodium nitrite (Sigma–Aldrich) and 10% (g/mL) sodium molybdatein (Sigma–Aldrich) in hydrochloric acid (0.5 M), is added into 1.0 mL of gelatin–dopamine solution (0.15%, g/mL) in DI water. The reaction solution is shaken for five minutes at 100 rpm at room temperature. The pH value and volume of the solution is adjusted to 7.0 and 5 mL, respectively, using sodium hydroxide solution