Development of a novel tissue adhesive using a naturally-derived small molecule

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Abstract

A citric acid derivative (CAD) with three active ester groups in a molecule was prepared in order to develop a novel tissue adhesive consisting of CAD and collagen (CAD-C glue). The bonding strength of CAD-C glue to porcine soft tissues had high value similar to the tearing strength of the original soft tissue. The cytotoxicity of CAD for L929 fibroblast was 10 times less cytotoxic than that of glutaraldehyde and formaldehyde that are used in gelatin-resorcinol-formaldehyde glue. This adhesive developed will be applied as bonding reagent for soft tissues.

Introduction

In order to bond tissue-tissue interface with high bonding strength, synthetic or semi-synthetic tissue adhesives, such as cyanoacrylate-based glue[1,2], gelatin-resorcinol-formaldehyde (GRF) glue[3], and other glues[4] have been developed and applied for biomedical field. However, GRF glue and cyanoacrylate-glue cause chronic inflammation and delay wound healing due to the residual reaction products[5] or degradation products[6] such as glutaraldehyde and formaldehyde. Therefore, fibrin glue[7] that is based on the polymerization reaction between fibrinogen and thrombin has been widely used in clinical applications. Fibrin glue has low cytotoxicity for human body and promotes wound healing, however, its low bonding strength to tissues limits the use in medical field. In order to adhere tissues with low cytotoxicity and high bonding strength, some researchers have tried to use various crosslinking reagents including genipin[8], diepoxy compounds[9] and carbodiimide[10]. These crosslinking reagents are reported to be much lower cytotoxicity than aldehyde compounds[11]. N-hydroxysuccinimide (NHS) - poly(L-glutamic acid) esters were also reported to crosslink gelatin and adhere tissues with low cytotoxicity[12]. However, these adhesives have not yet had sufficient bonding strength.

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Present study focused on the development of a novel tissue adhesive with low cytotoxicity and high bonding strength using a novel crosslinking reagent, NHS-citric acid ester (CAD). Bonding strength and cytotoxicity of a novel tissue adhesives consisting of CAD and collagen were evaluated.

Materials and Methods

CAD was synthesized by the reaction between citric acid (CA) and NHS in the presence of carbodiimide and was characterized by using ^{1}H-NMR (JEOL EX-500). Bonding strength measurement to tissues was carried out by following procedures reported by Iwata et al.[12]. A tissue from the porcine thigh was used for bonding strength test. A tissue adhesive (CAD-C glue) containing CAD and collagen (type I) was applied to one side of the tissue. The other tissue was then placed on the first layer. Bonding area was fixed at 2 x 2 cm^2. The bonding strength was measured at 25 ^{o}C using a tensile machine (EIKO TA-XT2i) at a test rate of 2 mm/s. GRF and fibrin (BOLHEAL®) glues were also used as control adhesives. Five samples were used for the same bonding strength test (n=5). Cytotoxicity test of CA, CAD, NHS and glutaraldehyde/formaldehyde (GA/FA) was performed by the culture of L929 fibroblasts. L929 cells were plated into a 96-well multiplate at a density of 1 x 10^4 cells/well in Eagle's MEM supplemented with L-glutamine and 10 % fetal bovine serum. Reagents for use in cytotoxicity test were dissolved in the same medium and sterilized by filtration. The reagent solutions were subsequently added to cells at various concentrations from 0.0001 to 100 mg/mL. Cell culture medium without additional reagents used as a control. After incubation for 3 days at 37 ^{o}C, 5 % CO_2, supernatant was removed from the 96-well multiplate and cells adhered were washed three times with phosphate buffer saline (PBS). The number of cells survive was determined by using Cell Counting Kit (Dojindo Laboratories, Kumamoto, Japan)[13]. All experiments were performed triplicate.

Results and Discussion

CAD was successfully obtained by the reaction between CA and NHS that is also a derivative of succinate in TCA cycle in the presence of carbodiimide. From the measurement of ^{1}H-NMR spectrum, the protons of methylene and succinimidyl groups were observed at 2.0 and 2.8 ppm, respectively. The esterification ratio determined from the relative areas of

these peaks was 3.6, indicating that three carboxyl groups in CA were completely esterified.

Then, a tissue adhesive consisting of CAD and collagen were prepared using the CAD as a crosslinking reagent. Measurement of bonding strength was carried out using porcine thigh specimens. Tissue adhesives for bonding strength test were CAD-C, GRF, and fibrin glue. An original porcine thigh specimen was also used as a control. CAD-C glue had greatest bonding strength (157.2 g/cm^2) similar to the tearing strength of an original porcine thigh specimen (191.4 g/cm^2) and bonding strength of GRF glue (158.4 g/cm^2). The bonding strength of CAD-C glue was higher than that of fibrin glue (104.5 g/cm^2). It is known that active ester groups can react with amino groups in proteins. Therefore, active ester groups of CAD can also react with amino groups of lysine or hydroxylysine residues within collagen or other extracellular matrix component not only in the CAD-C glue but also in porcine thigh, resulting in the formation of amide bond. This covalent bonding formation causes the high bonding strength of CAD-C glue to soft tissues. The bonding strength of CAD-C glue also increased with an increase of CAD concentration. Increased boding strength of CAD-C glue to tissues revealed that the increased covalent crosslinking density between CAD, collagen within glue and extracellular matrix components of porcine thigh.

Cytotoxicity of CA, CAD, NHS and GA/FA which is the component of GRF glue increased with an increase of concentrations of these reagents tested. IC_{50} of CAD and CA was 0.58 and 0.54 mg/mL, respectively, indicating that these reagents were low cytotoxicity. While, high cytotoxicity (IC_{50} : 0.04 mg/mL) was observed when GA/FA was added to L929 fibroblasts. The cytotoxicity of GA/FA was 10-times higher than that of CAD. It was reported that cytotoxicity test of chemical substances *in vitro* had good correlation with *in vivo* experiment of chemical substances[9]. Remaining aldehyde compounds in tissue adhesive show cytotoxicity for surrounding tissues and delay wound healing. Due to the hydrolysis from CAD to CA, CAD will show low cytotoxicity for tissues even if it will remain in the adhesive *in vivo*. Thus, CAD-C glue achieved high bonding strength as well as low cytotoxicity.

Conclusion

A novel tissue adhesive (CAD-C glue) consisting of collagen and CAD that was CA derivative with three active ester groups was developed. Adhesive strength of CAD-C glue to porcine soft tissue was similar to the tearing strength of the original tissues. The

cytotoxicity of CAD for L929 fibroblast was 10-fold lower than that of glutaraldehyde and formaldehyde that are the component of gelatin-resorcinol-formaldehyde glue applied in clinical use. This adhesive developed will be applied to be a bonding reagent for not soft tissues.

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