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Rapidly curable chitosan–PEG hydrogels as tissue adhesives for hemostasis and wound healing

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ABSTRACT

Chitosan–poly(ethylene glycol)–tyramine (CPT) hydrogels were rapidly formed in situ using horseradish peroxidase and hydrogen peroxide to explore their performance as efficient tissue adhesives. A poly(ethylene glycol) modified with tyramine was grafted onto a chitosan backbone to enhance the solubility of the chitosan and to crosslink into three-dimensional networks. The elastic modulus of the hydrogels could be controlled by changing the crosslinking conditions, and the mechanical strength influenced the tissue adhesiveness of the hydrogels. The hydrogels showed the adhesiveness ranging from 3- to 20-fold that of fibrin glue (Greenplast®). The hemostatic ability of the hydrogels was evaluated on the basis that bleeding from liver defects was significantly arrested by the combined effect of the adhesiveness of the hydrogels and the hemostatic property of the chitosan materials. The enzymatic crosslinking method enabled the water-soluble chitosan to rapidly form hydrogels within 5 s of an incision into the skin of rats. Histological results demonstrated that the CPT hydrogels showed superior healing effects in the skin incision when compared to suture, fibrin glue and cyanoacrylate. By 2 weeks post-implantation, the wound was completely recovered, with a newly formed dermis, due to the presence of the CPT hydrogels in the incision. These results suggest that the in situ curable chitosan hydrogels are very interesting and promising tissue adhesive devices for biomedical applications.

1. Introduction

Tissue adhesives have attracted rapidly growing interest as sealants, hemostatic agents and non-invasive wound-closure devices [1]. The adhesives are required to perform a variety of functions, including sealing leaks, stopping bleeding, binding tissues and preferably facilitating a healing process [2]. Adhesion to biological tissues is a highly challenging task because the adhesive materials should exhibit suitable physical properties (elasticity, tensile and adhesive strength), biocompatibility and biodegradability in contact with physiological fluids. Fibrin glues are widely used as biological tissue adhesives in surgical practices, but sometimes their mechanical property is not sufficient and they are required to be applied on dry substrates [3,4]. Cyanoacrylates are a class of synthetic glues that rapidly solidify upon contact with weak bases (water or blood) and guarantee a high degree of adhesiveness [5]. However, the acrylic derivatives exhibit toxicity, due to aldehydes, which are the degradation products of the glues [6–8]. There have been considerable efforts to develop various synthetic-material-based tissue adhesives (acrylates and poly(ethylene glycol) (PEG) hydrogels), biological adhesives (fibrin glues, polysaccharides and proteins) and hybrid systems [9–13].

One of the prime candidates, chitosan has been used as a wound dressing material due to its superior tissue- or mucoadhesive property, hemostatic activity, low toxicity, relevant biodegradability and anti-infection activity [14–16]. Chitosan is a cationic polysaccharide and its adhesive properties are mainly based on ionic interactions with tissues or mucus layers [17,18]. Low-molecular-weight chitosan is particularly known to facilitate closer interaction with the surface of the epithelial cells [14,19]. Despite the advantages, the rigid crystalline structure of chitosan makes it hard to dissolve in water, and this has partially retarded its potential for such application [20]. Modification of the chitosan with PEG can enhance the water solubility of chitosan and permit the formation of chitosan-based hydrogels by crosslinking of the PEG.

Although in situ forming hydrogels have been suggested as ideal injectable biomaterials, certain properties, like weak mechanical strength, rapid dissolution and cytotoxicity of the hydrogels, need to be considered. Recently, enzyme-mediated in situ crosslinkable hydrogels have received a great deal of attention in tissue engineering because of their tunable mechanical property, rapid gelation time and low toxicity, and the mild crosslinking conditions [21–25]. Park and colleagues reported in situ formation of hydrogels based on tyramine-conjugated Tetronic® or gelatin–PEG via

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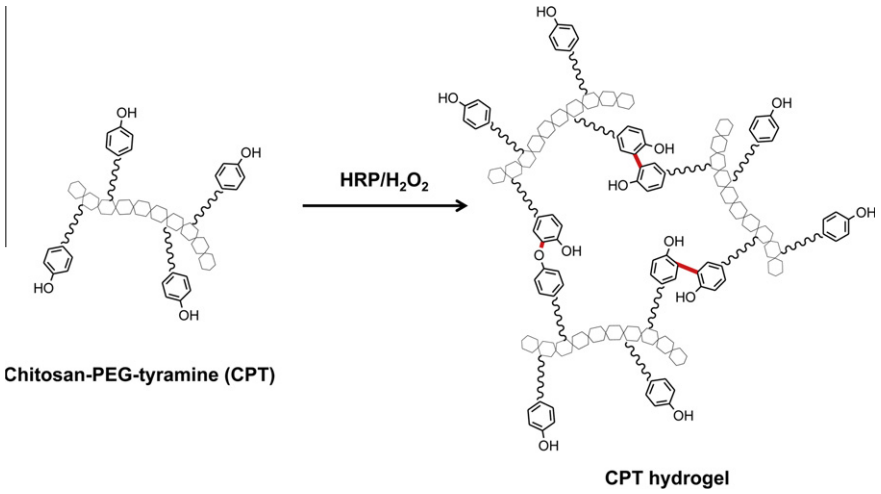

Fig. 1. Schematic representation of in situ gel formation of CPT conjugates using HRP and H_2O_2.

enzymatic oxidative reactions using horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) [23–26]. HRP is a hemoprotein that catalyzes the conjugation of phenol and aniline derivatives with decomposed H_2O_2 molecules [27]. The enzymatically crosslinked hydrogels showed excellent bioactivities and tunable physicochemical properties, suggesting that this type of hydrogel has great potential for use as an injectable material for tissue regenerative medicine and various biomedical applications.

In this study, we report on enzyme-triggered in situ formation of hydrogels based on chitosan as a tissue adhesive material for hemostasis and wound healing. For the formation of the hydrogels, chitosan was grafted with tyramine-modified PEGs and the tyramines were crosslinked by HRP and H_2O_2 as shown in Fig. 1. The enzymatic crosslinking enabled the water-soluble chitosan to rapidly form hydrogels, which stably adhered to the wound site for a desired period of time. The hydrogels were characterized in terms of their physicomechanical properties, such as gelation time, elastic moduli and adhesive strengths, under various conditions. The hemostatic and adhesive properties of the hydrogels as well as the wound healing capability were also evaluated in vivo.

2. Materials and methods

2.1. Materials

Chitosan (low molecular weight, 75–85% deacetylated), poly(ethylene glycol) (4000 g mol^{-1}), HRP (units per mg solid (using pyrogallol)), hydrogen peroxide, 4-dimethylamino pyridine (DMAP) and p-nitrophenylchloroformate (PNC) were purchased from Sigma–Aldrich (St. Louis, MO). Tyramine (TA) was purchased from Acros Organics. Triethylamine (TEA) and aluminum oxide were obtained from Kanto Chemical Co. and Strem Chemicals, respectively. Fibrin glue kit (Greenplast®) was purchased from Green Cross Co. and *n*-butyl-2-cyanoacrylate adhesive (Histoacryl®) was obtained from Tissueseal, LLC (Ann Arbor, MI, USA). For cell culture, Dulbecco's modified Eagle's medium (high glucose), fetal bovine serum, trypsin/ethylenediaminetetraacetic acid, penicillin–streptomycin and phosphate-buffered saline (PBS, pH 7.4) were obtained from Gibco BRL (Carlsbad, CA). Fluorescein diacetate and ethidium bromide were purchased from Sigma–aldrich. A Cell Counting Kit-8 was purchased from Dojindo (Kumamoto, Japan). All other chemicals and solvents were used such without further purification.

2.2. Synthesis of chitosan–poly(ethylene glycol)–tyramine (CPT) conjugates

The CPT conjugates were synthesized according to the previously reported method [23]. Amine-reactive PEG (PNC–PEG–PNC) was prepared by activating the hydroxyl groups of PEG with excess of PNC. The activated PEG was reacted with TA and subsequently with chitosan, to form the CPT conjugates (Fig. 2). Briefly, PEG (10 g, 5 mmol of hydroxyl groups) was dissolved in methylene chloride (MC, 100 ml) at room temperature under a nitrogen atmosphere.

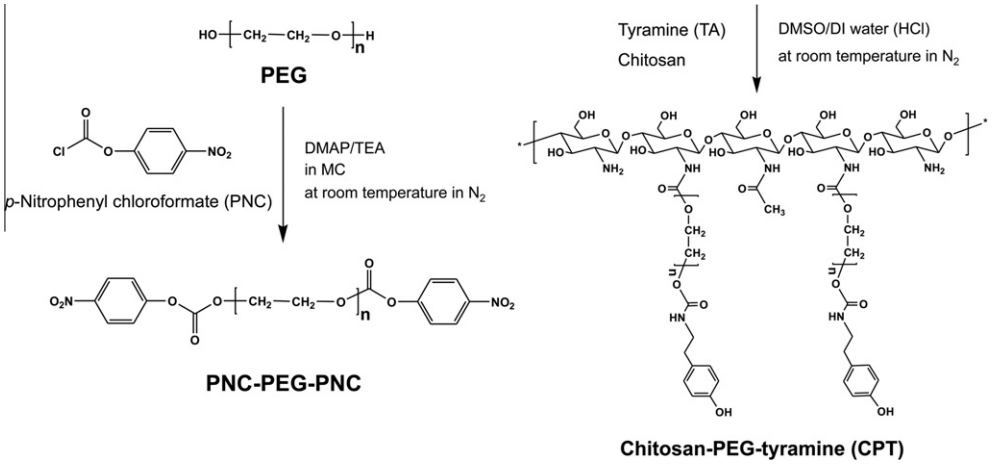

Fig. 2. Synthetic route of the CPT conjugates. Preparation of (a) PNC–PEG–PNC and (b) the conjugation of the PNC–PEG–TA with chitosan.

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