Biomaterials 67 (2015) 11–19

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Rapidly light-activated surgical protein glue inspired by mussel adhesion and insect structural crosslinking

Eun Young Jeon [a, 1], Byeong Hee Hwang [a, b, 1], Yun Jung Yang [a], Bum Jin Kim [a], Bong-Hyuk Choi [a], Gyu Yong Jung [c], Hyung Joon Cha [a, *]

[a] Department of Chemical Engineering, Pohang University of Science and Technology, Pohang 790-784, South Korea
[b] Division of Bioengineering, Incheon National University, Incheon 406-772, South Korea
[c] Department of Plastic Surgery, College of Medicine, Dongguk University, Gyeongju 780-714, South Korea

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form
4 July 2015
Accepted 10 July 2015
Available online 14 July 2015

Keywords:
Mussel adhesive protein
Protein adhesive
Tissue adhesive
Light-activated hydrogel
Dityrosine crosslinks

ABSTRACT

Currently approved surgical tissue glues do not satisfy the requirements for ideal bioadhesives due to limited adhesion in wet conditions and severe cytotoxicity. Herein, we report a new light-activated, mussel protein-based bioadhesive (LAMBA) inspired by mussel adhesion and insect dityrosine crosslinking chemistry. LAMBA exhibited substantially stronger bulk wet tissue adhesion than commercially available fibrin glue and good biocompatibility in both *in vitro* and *in vivo* studies. Besides, the easily tunable, light-activated crosslinking enabled an effective on-demand wound closure and facilitated wound healing. Based on these outstanding properties, LAMBA holds great potential as an ideal surgical tissue glue for diverse medical applications, including sutureless wound closures of skin and internal organs.

1. Introduction

A sutureless wound closure is essential to simplify surgical procedures and improve patient care quality. Traditionally, mechanical fasteners like sutures and staples have been used in 60% of rejoining tissues [1]. However, they are not always suitable, especially for preventing the leakage of air or bodily fluids and for suturing friable tissues and non-accessible organs. They can also cause severe tissue damage due to localized stress and need suture removal. Therefore, tissue adhesives are being pursued to overcome the obstacles and promote wound healing without penetration [2]. Despite intensive studies, a perfect bonding material has not yet been developed, and current available options have significant limitations. For example, cyanoacrylates, a chemically derived representative, have toxicity [3]. Biologically derived fibrin glue has limited tissue adhesion [4]. Moreover, most developed glues are easily washed out in a wet environment due to poor mechanical properties or long curing times [5].

Fortunately, nature has provided a captivating source for ideal tissue glue, namely mussel adhesive proteins (MAPs) [6]. Mussels can stably fix their body to wet surfaces with byssal threads. To understand mussel adhesion mechanisms, six distinct MAPs, i.e., foot protein type 1 (fp-1) to foot protein type 6 (fp-6), have been identified from byssal plaques, and 3,4-dihydroxy-phenylalanine (DOPA) residues in natural MAPs have been considered as a key factor for strong underwater adhesion [7,8,10]. Previously, to overcome limited applications due to the extreme difficulty in preparing natural MAPs, hybrid-type recombinant MAP was newly designed and successfully mass-produced in bacterial system [8,9]. This recombinant MAP has proven to be flexible, biodegradable, biocompatible, and strongly adhesive on various surfaces [9,11]. However, due to the intrinsic inabilities of a bacterial system such as lack of post-translational modifications, enzyme-mediated DOPA modification step is required to prepare DOPA-containing recombinant MAP. It is essential to address current challenges of low modification yield and DOPA-containing MAP instability [12,13]. Therefore, a direct use of recombinant MAP without modification would be a facile and economical alternative for MAP-based medical applications such as surgical wound closure.

Herein, to develop a rapidly acting surgical protein glue creating stable adhesive and cohesive bonds without DOPA modification, we utilized a dityrosine photo-crosslinking strategy in recombinant

* Corresponding author.
E-mail address: hjcha@postech.ac.kr (H.J. Cha).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2015.07.014
0142-9612/

MAP. In insect structural proteins including resilins of dragonfly wings [14], fibroins of silk worms [15], and locust cuticles [16], dityrosine crosslinks are expected to occur spontaneously between tyrosine residues via photo-oxidation reaction. Because dityrosine crosslinks are known to confer mechanical and conformational stability and elasticity to protein chains [17], their introduction into tyrosine-rich (approximately 20 mol%) MAP may significantly increase structural support and adhesive properties as a very stable bridge. Additionally, the use of a visible light is anticipated for controllable, safe, and rapid wound closures. Therefore, we evaluated the novel concept (Fig. 1) of a light-activated mussel protein-based bioadhesive (LAMBA) as an ideal tissue glue via *in vitro* and *in vivo* studies.

2. Materials and methods

2.1. Preparation of recombinant hybrid MAP

Recombinant hybrid MAP fp-151, which is comprised of six fp-1 decapeptide repeats at both the N- and C-termini of fp-5, was produced in an *Escherichia coli* system as previously reported [9]. In brief, transformed *E. coli* BL21 (DE3) cells were cultured in 5 L Luria–Bertani (LB) medium containing 50 μg/mL ampicillin (Sigma) at 37 °C and 300 rpm. At an optical density of 0.4–0.6 at 600 nm, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma) was added to the medium to induce the expression of recombinant MAP. The cells were subsequently incubated for another 8 h at 37 °C and 300 rpm. After centrifugation of the broth at 9000 × g for 10 min at 4 °C, the cell pellets were suspended in 15 mL lysis buffer (10 mM Tris–HCl and 100 mM sodium phosphate; pH 8.0) per gram wet weight. The cells were lysed with constant cell disruption systems (Constant Systems) at 20 kpsi. The lysates were centrifuged at 9000 × g for 20 min at 4 °C, and isolated inclusion bodies were treated with Triton X-100 washing buffer (1% (v/v) Triton X-100 (Sigma), 1 mM EDTA, and 50 mM Tris–HCl; pH 8.0) with 10 mM phenylmethanesulfonyl fluoride (PMSF; Sigma) and 1 mg/mL lysozyme (Bio Basic) and agitated overnight. After centrifugation, the inclusion bodies were resuspended in Triton X-100 washing buffer and then resuspended in 40 mL of 25% (v/v) acetic acid per gram wet weight to extract recombinant MAP with relatively high purity (~97%) [9]. The extracted solution was centrifuged at 9000 × g for 20 min at 4 °C, and the supernatant was collected, dialyzed in deionized water (DW), and then lyophilized.

2.2. Photochemical crosslinking for fabrication of LAMBA hydrogels

To couple two adjacent tyrosine residues into a dityrosine adduct, we employed a well-established photo-oxidative reaction involving the activation of a ruthenium complex, Ru(II)bpy$_3^{2+}$, with an electron acceptor (e.g., sodium persulfate; SPS) in the presence of blue light [18]. Briefly, fresh stock solutions of 50 mM Ru(II)bpy$_3^{2+}$ (Sigma) and 500 mM SPS (Sigma) were prepared via dissolution in DW, respectively. Mixtures of recombinant MAP, 2 mM Ru(II)bpy$_3^{2+}$, and 10–30 mM SPS in 200 mM sodium acetate buffer (pH 5.5) were dispensed into Teflon molds or tip-removed 1 mL syringes and irradiated for 60 s at room temperature with an LED dental curing lamp (460 nm, 1200 mW/cm^2; FORZA4) from a distance of 20 mm.

2.3. Detection of dityrosine links in LAMBA hydrogels

The presence of dityrosine and dityrosine-like compounds in fabricated LAMBA hydrogel after the photochemical crosslinking reaction was evaluated via the fluorescence detection of acid hydrolysates [19]. These compounds have particular emissions at 410 nm when excited at 315 nm. To induce the full hydrolysis of amide bonds, 10 μL hydrogel sample with 2 mg MAP was reacted with 1 mL of 6 N HCl in a sealed 1.5 mL centrifuge tube in a heat block at 105 °C for 2 h. Then, 100 μL of acid hydrolysis product was transferred to a new 1.5 mL centrifuge tube and neutralized with 5 M NaOH. Then, 100 mM Na_2CO_3–$NaHCO_3$ buffer (pH 9.9) was added to the tube to a 1 mL final volume. To confirm the crosslinking density of the hydrogels made with different SPS concentrations, the fluorescence spectra of the samples were measured. The yields of the dityrosine and dityrosine-like products in the hydrogels were quantified according to the fluorescence concentration standard curve of purified dityrosine.

2.4. Surface morphology analysis of LAMBA hydrogel

The photochemically crosslinked hydrogels were equilibrated in DW for 24 h and then lyophilized for 2 days. The swollen, freeze-dried samples were cross-sectioned, mounted on an aluminum substrate, and sputter-coated with gold for 40 s. The internal structures of the gel specimens were investigated via scanning electron microscopy (SEM; JEOL) at 5.0 kV.

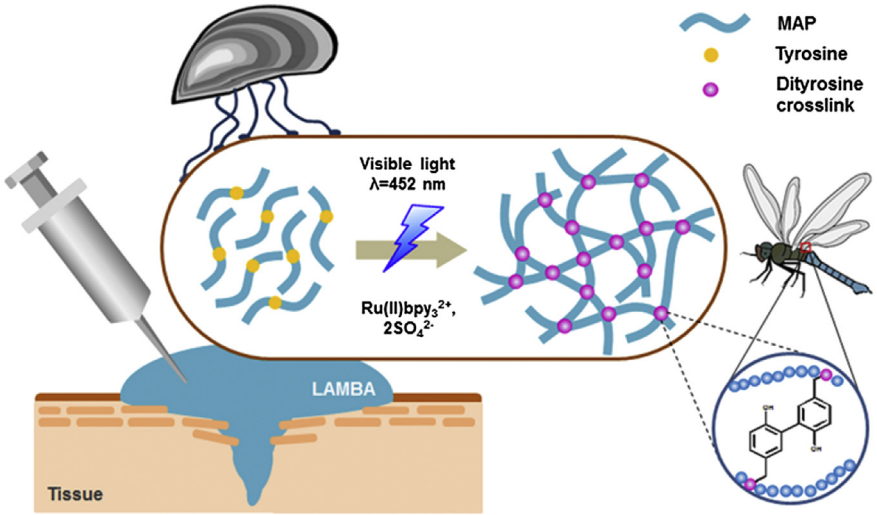

Fig. 1. Schematic representation of recombinant MAP-based bioadhesive formation via dityrosine bonds using visible light.