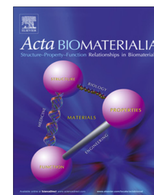
Acta Biomaterialia 10 (2014) 2539–2550

Contents lists available at ScienceDirect

Acta Biomaterialia

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



Enzymatic conjugation of a bioactive peptide into an injectable hyaluronic acid–tyramine hydrogel system to promote the formation of functional vasculature

Li-Shan Wang, Fan Lee, Jaehong Lim, Chan Du, Andrew C.A. Wan, Su Seong Lee, Motoichi Kurisawa *

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669, Singapore

ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form 30 January 2014
Accepted 11 February 2014
Available online 21 February 2014

Keywords:
Hydrogel
Vascularization
RGD
Hyaluronic acid
Functionalization

ABSTRACT

In this study, one-step enzyme-mediated preparation of a multi-functional injectable hyaluronic-acid-based hydrogel system is reported. Hydrogel was formed through the in situ coupling of phenol moieties by horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2), and bioactive peptides were simultaneously conjugated into the hydrogel during the gel formation process. The preparation of this multi-functional hydrogel was made possible by synthesizing peptides containing phenols which could couple with the phenol moieties of hyaluronic-acid–tyramine (HA–Tyr) during the HRP-mediated crosslinking reaction. Preliminary studies demonstrated that two phenol moieties per molecule resulted in a consistently high degree of conjugation into the HA–Tyr hydrogel network, unlike the one modified with one phenol moiety per molecule. Therefore, an Arg–Gly–Asp (RGD) peptide bearing two phenol moieties (phenol$_2$–poly(ethylene glycol)–RGD) was designed for conjugation to endow the HA–Tyr hydrogel with adhesion signals and enhance its bioactivities. Human umbilical vein endothelial cells (HUVECs) cultured on or within the RGD-modified hydrogels showed significantly different adhesion behavior, from non-adherence on the HA–Tyr hydrogel to strong adhesion on hydrogels modified with phenol$_2$–poly(ethylene glycol)–RGD. This altered cell adhesion behavior led to improved cell proliferation, migration and formation of capillary-like network in the hydrogel in vitro. More importantly, when HUVECs and human fibroblasts (HFF1) were encapsulated together in the RGD-modified HA–Tyr hydrogel, functional vasculature was observed inside the cell-laden gel after 2 weeks in the subcutaneous tissue. Taken together, the in situ conjugation of phenol$_2$–poly(ethylene glycol)–RGD into HA–Tyr hydrogel system, coupled with the ease of incorporating cells, offers a simple and effective means to introduce biological signals for preparation of multi-functional injectable hydrogels for tissue engineering application.

© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Hydrogels are widely used as scaffolds for tissue engineering [1]. The aqueous three-dimensional (3-D) environment within a hydrogel is not only suitable for the encapsulation of cells, but is also capable of presenting biological signals in the form of bioactive peptides grafted or incorporated into the gel network. Presentation of bioactive peptides aims to recapitulate the extracellular matrix (ECM) of the tissue to be regenerated and/or stimulate the encapsulated cells with specific biological signals. There are two main approaches to introduce bioactive peptides into a hydrogel network. The first is a step-wise approach in which peptide-conjugated synthetic/natural polymers are first synthesized and subsequently crosslinked to form a hydrogel [2–7]. The second is a one-step preparation approach in which peptides containing reactive groups are conjugated into the hydrogel network during the crosslinking reaction in situ [8–11]. For example, the enzymatic activity of Factor XIIIa was employed to covalently incorporate a variety of exogenous oligopeptide mimetics or pairs of them at controllable concentrations within the fibrin network during coagulation [10]. This in situ conjugation provided a simple and effective approach to endow fibrin with adhesion and morphogenetic signals that are not naturally present within the material. At the same time, an extensive alteration to the inherent properties of the materials was avoided during the modification process, unlike its step-wise counterpart where the modification is directly made to the polymers.

* Corresponding author. Tel.: +65 6824 7139; fax: +65 6478 9083.
E-mail address: mkurisawa@ibn.a-star.edu.sg (M. Kurisawa).

http://dx.doi.org/10.1016/j.actbio.2014.02.022
1742-7061/© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Hyaluronic acid (HA), a non-sulfated glycosaminoglycan found in the ECM, has been widely used to form hydrogels for biomedical applications. HA is most abundant during early embryogenesis and plays critical roles in regulating the angiogenic process and wound healing [12,13]. Although naturally derived, it can be commercially produced using microbial fermentation. Therefore it is a promising material for making tissue engineering scaffolds due to its low immunogenicity and biodegradability. However, HA is known to resist cell adhesion, limiting the use of HA-based gels for some tissue engineering applications. Several methods have been developed to enhance the cell-adhesive property of hydrogels, including the incorporation of Arg–Gly–Asp (RGD) peptide [4,9,14] into the gel matrices or ECM components such as fibronectin [15] collagen [16] and fibrin [17]. The presence of RGD, a well-known cell adhesion ligand found in fibronectin, is found not only to promote integrin-mediated cell adhesion, but also to enhance the activity of endothelial cells [18–21]. As insufficient vascularization remains a major challenge to overcome in order to form thicker and more complex tissues, there have been numerous attempts to promote the formation of the vascular network in the engineered constructs for effective tissue engineering by incorporating or grating ECM proteins, ECM-derived peptides or signaling protein including the RGD peptide [2,22–30].

Previously we described an injectable hyaluronic acid–tyramine (HA–Tyr) hydrogel system formed by enzyme-mediated crosslinking of tyramine moieties using horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2). The HRP-mediated crosslinking reaction has become an attractive method to form hydrogels in situ due to its substrate specificity, efficiency and lack of involvement of toxic crosslinkers [17,31–39]. The HRP-mediated coupling of phenol moieties could further be utilized for the in situ conjugation of bioactive peptides into the hydrogel network. Indeed, Park et al. recently demonstrated the conjugation of an endothelial cell binding peptide, Ser–Val–Val–Tyr–Gly–Leu–Arg, into a gelatin–PEG–tyramine (GPT) hydrogel by HRP-mediated crosslinking [8]. This endothelial cell binding peptide terminates with a tyrosine residue which readily forms C–C or C–O bonds with the tyramine moieties of GPT during the crosslinking reaction. The modified cell-free GPT hydrogel was able to influence the activity of endothelial cells in surrounding tissues and enhance angiogenic activity and cell migration to the construct.

In this paper, a detailed study was first performed to examine the coupling efficiency of phenol-containing RGD peptides into the HA–Tyr hydrogel network using two model compounds, one bearing a single phenol moiety while the other bearing two phenol moieties. Having confirmed that a molecule containing two phenol moieties had a more consistent coupling efficiency and resulted in hydrogels with superior rheological properties, we proceeded to design an RGD peptide bearing two phenol moieties per molecule (phenol$_2$–PEG–RGD; PEG = poly(ethylene glycol)). The phenol$_2$–PEG–RGD was readily conjugated into HA–Tyr hydrogels during gel formation process by the HRP-mediated crosslinking reaction. The RGD-modified hydrogels (HA–Tyr–RGD) were then characterized in terms of rheology, water uptake and cell adhesion, proliferation and migration. Furthermore, human umbilical vein endothelial cells (HUVECs) and human fibroblasts (HFF1) were mixed together with phenol$_2$–PEG–RGD and HA–Tyr conjugates to form a cell-laden HA–Tyr–RGD hydrogel for the purpose of promoting a capillary-like network in the hydrogel. The cell-laden HA–Tyr–RGD hydrogels were found not only to promote a capillary-like network in the hydrogel in vitro, but also to result in functional vasculature 2 weeks after the gel injection into the subcutaneous tissue. We believe that this one-step preparation of the injectable HA–Tyr hydrogel system with additional peptide motifs via HRP-mediated crosslinking provides a new platform to customize the gel with multiple bioactive motifs tailored to the tissue of interest.

2. Materials and methods

2.1. Materials

HA (90 kDa) was kindly donated by JNC Corp. (Tokyo, Japan). Type I collagenase (246 units mg^{-1}), hyaluronidase from bovine testes and Triton X-100 were purchased from Sigma–Aldrich. Hydrogen peroxide was from Lancaster and HRP, 100 units mg^{-1} was from Wako Pure Chemical Industries. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, Quant-iT™ PicoGreen ® dsDNA Reagent and Kits and CellTracker Green CMFDA were provided by Life Technologies (Singapore). An actin cytoskeleton and focal adhesion staining kit containing vinculin monoclonal antibody, tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin and 4′,6-diamidino-2-phenylindole (DAPI) and human nuclei antibody (MAB 1281) were provided by Millipore (Singapore). Mouse monoclonal anti-CD31 antibody (ab9498) was obtained from Abcam. An anti-mouse HRP-DAB cell and tissue staining cell was purchased from R&D systems (USA). A Pierce™ BCA protein assay kit was obtained from Thermo Scientific (Singapore).

2.2. Synthesis and characterization of phenol$_2$–PEG–RGD

The phenol$_2$–PEG–RGD was synthesized by using an automatic synthesizer Titan 357 (AAPPTEC). 50 mg of ChemMatrix® resins (0.48 mmol g^{-1}) was swelled in 1 ml of *N*-methylpyrrolidone (NMP) for 5 min in a reaction vessel (RV). With the liquid drained, 1 ml of 20% piperidine in NMP (v/v) was added and the RV was vortexed for 2 min. The liquid was drained and 1 ml of fresh solution (20% piperidine in NMP, v/v) was added. The RV was vortexed for another 10 min. The resulting beads were thoroughly washed with NMP (1 ml × 2), methanol (1 ml × 2) and dichloromethane (DCM, 1 ml × 2), successively. With the resulting resins swelled with NMP for 15 min, Fmoc-D(OtBu)-OH (2.5 equiv, 0.2 M solution in NMP) was added to the RV, as well as *N,N,N′,N′*-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU, 2.5 equiv, 0.2 M solution in NMP) and *N,N*-diisopropylethylamine (DIEA, 5.0 equiv, 0.5 M in NMP). The resulting mixture was vortexed for 45 min. With the liquid drained, the resulting beads were thoroughly washed with NMP (1 ml × 3). The coupling step was repeated until the desired structure attained on beads, i.e., HPA–K(Mtt)–PEG2(13 atm)–R(Pbf)–G–D(OtBu). Then, selective deprotection of the 4-methyltrityl (Mtt) group was performed by reaction with 2 ml of trifluoroacetic acid/triisopropylsilane/DCM (TFA/TIS/DCM, 3/3/94, v/v/v) for 2 min, 5 min and 30 min successively, using a fresh aliquot each time. Finally, another group of 3-(4-hydroxyphenyl) propionic acid (HPA) was coupled to the exposed amine group at the residue of K. The resins were washed with NMP (1 ml × 3) and transferred in a 4 ml reactor equipped with a filter, using DCM (2 ml × 3). After the resins were dried under reduced pressure for 2 h, the peptide was cleaved in a cleavage cocktail of TFA–water–TIS (1.5 ml, 94/3/3, v/v/v) for 2 h on a 180° shaker, while all the acid-labile protective groups in the residues were also detached. The solution was collected and concentrated in a continuous flow of nitrogen and the crude peptides were precipitated in diethylether. The resulting white solid was then purified to >98% in purity by preparative high-performance liquid chromatography (HPLC; Gilson) on a C18 reversed phase preparative column (Kromasil®, 21.2 mm × 250 mm) using water and acetonitrile with 0.1% TFA as the mobile phase. The absorbance of phenol$_2$–PEG–RGD (0.1 mM) was measured at 276 nm using an ultraviolet–visible (UV–vis) spectrophotometer (U-2810, Hitachi, Japan) and was compared to that of HPA (0.1 mM).

Download English Version:

https://daneshyari.com/en/article/10159092

Download Persian Version:

https://daneshyari.com/article/10159092

Daneshyari.com