In Situ Forming Gelatin-Based Bioadhesive and Sprayable Hydrogels for Skin Regeneration

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Statement of Purpose: Wound healing is a complex and dynamic process that restores cellular structures and tissue layers. To promote wound healing, many attempts using various types of scaffolds have been made so far and biomimetic hydrogels carrying cells/growth factors are regarded as an attractive candidate for skin regeneration.

In this study, we report in situ forming gelatin-based bioadhesive hydrogels that can deliver human dermal fibroblasts (hDFBs) and basic fibroblast growth factor (bFGF) for promoted wound healing. This hydrogel system, which is enzymatically cross-linkable, can offer great benefits as an easy-to-use delivery vehicle. Particularly, various hydrogel properties such as gelation time and mechanical strength can be modulated by adjusting hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP). The purpose of this study is to investigate stiffness-dependent wound healing effects of gelatin-based hydrogels carrying hDFBs and bFGF.

Methods: The HRP-reactive gelatin derivative (GH) was synthesized by conjugating hydroxyphenyl propionic acid (HPA) to gelatin backbones. The chemical structure and phenolic content of the GH were characterized by ^{1}H NMR and UV-vis spectrometer, respectively. As shown in Figure 1, the GH hydrogels were fabricated by simply mixing with HRP and H_2O_2. Their physico-chemical properties such as gelation time, mechanical strength, and degradation behavior were systemically studied by varying HRP and H_2O_2. bFGF release profiles from the GH hydrogels with different stiffness (2.2 kPa, soft; 10.1 kPa, hard) were confirmed using a sandwich ELISA method. In vitro cell study was carried out by 3D culture of hDFBs in bFGF incorporated GH hydrogels, followed by a PicoGreen assay.

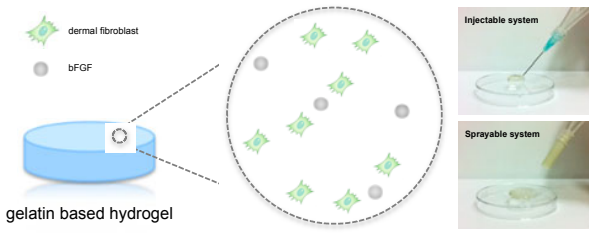

Figure 1. Schematic representation of in situ formed GH hydrogels for delivery of hDFBs and bFGF.

Results: The ^{1}H NMR spectra clearly showed the characteristic peaks corresponding to phenol groups of the gelatin derivative, which appear at 6.8-7.1 ppm. In addition, the amount of conjugated HPA was quantitatively measured to be approximately 150 μmol per 1 g of GH. The gelation time of the GH hydrogels was decreased with increasing the HRP concentrations, ranging from 5 sec to 3 min. The mechanical strength of the hydrogels was improved with increasing the H_2O_2 concentration due to increased cross-linking density (2.2-10.1 kPa). It was found that the hydrogels were completely degraded within 4 days after collagenase treatment (0.38 U/mL), showing different degradation rates according to cross-linking density. The incorporated bFGF was slowly released out from stiffer hydrogels for 1 week. When cultured for 15 days, the encapsulated hDFBs tended to be more proliferative in less stiff hydrogels, indicating that a lower cross-linking density is advantageous over transport of nutrients (Figure 2). The hDFBs proliferation was enhanced when cultured in the bFGF incorporated hydrogels with less stiffness.

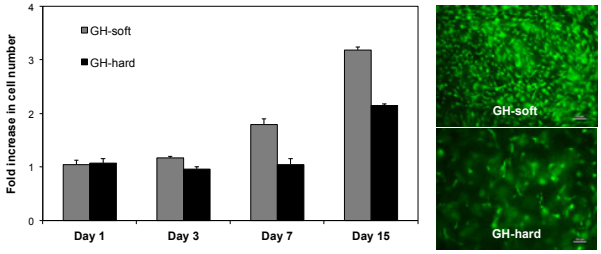

Figure 2. 3D hDFBs proliferation and fluorescence images of hDFBs cultured in soft (2.2 kPa) and hard (10.1 kPa) GH hydrogels.

Conclusions: We have developed the gelatin-based bioadhesive hydrogels that can be formed in situ by enzymatic cross-linking for skin regeneration. The obtained results demonstrated that various hydrogel properties could be controlled by adjusting cross-linking rate or degree of the hydrogels. In addition, it was confirmed that the less stiff GH hydrogels is more suitable for enhanced proliferation of hDFBs. Encapsulation of bFGF and hDFBs into the less stiff GH hydrogels enabled to promote hDBF proliferation effectively. Therefore, we expect that the gelatin-based bioadhesive hydrogels can be useful as a sprayable biomimetic scaffold to carry bFGF and hDFBs for wound healing promotion.

References:

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