In Situ Forming Gelatin-Poly(Ethylene Glycol)-Tyramine Hydrogels *via* Dual Enzyme of Horseradish Peroxidase and Gluose Oxidase

Bae Young Kim, Jin Woo Bae, Ki Dong Park*
Department of Molecular Science and Technology, Ajou University, Suwon, Republic of Korea
(*kdp@ajou.ac.kr)

Statement of Purpose: *In situ* forming hydrogel is an atrractive material platform for a wide range of biomedical applications. Among various *in situ* forming hydrogels, enzymatically cross-linked hydrogels *via* a horseradish peroxidase (HRP)-catalyzed reaction are of particular interest because their hydrogel properties are fairly tunable by combinatorial mixing of constituents including phenol-rich polymer, HRP and H_2O_2.[1] In previous research, direct addition of H_2O_2 molecules dissolved in aqueous solution into the oxidative reaction has been the only way to supply the H_2O_2 necessary for achieving enzymatic *in situ* hydrogelation.[2] Herein, we developed *in situ* cross-linkable gelatin-based hydrogels *via* HRP- and Glucose oxidase (GOx)-mediated reaction.

Methods: The gelatin-poly(ethylene glycol)-tyramine (GPT) conjugate was synthesized by conjugating tyraimine to gelatin backbone using the PEG as a hydrophilic linker. *In situ* GPT hydrogels were prepared by simply mixing two GPT solutions with different compositions, each containing HRP/GOx and glucose (Figure 1). We indirectly supplied H_2O_2 to the HRP-catalyzed hydrogelation system using a GOx as an oxidoreductase that catalyzes the oxidation of glucose to H_2O_2 and glucono-δ-lactone. Catalytic activity of the enzymes was evaluated using a colorimetric ABTS assay. Gelation time and elastic modulus of the hydrogels were investigated with different concentration of GOx and glucose. In addition, proteolytic degradation rate of the hydrogels was evaluated *in vitro* using 0.2 mg/mL of collagense. *In vitro* cell studies were carried out using hDFBs in the hydrogel to evaluate the effect of contained GOx on cell viability and proliferation.

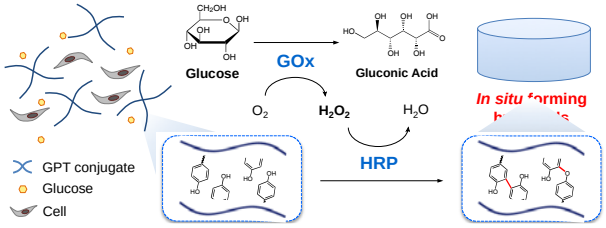

Figure 1. Schematic route for the HRP-and GOx-mediated *in situ* hydrogelation of GPT conjugate

Results: The GPT conjugate was successfully synthesized with a phenolic content of 146.6 μmol in 1 g of gelatin-derivatives. The dual enzyme-triggered were prepared by simple mixing of the polymer solutions dissolved in enzymes and glucose solutions. The gelation times decreased from around 600 s to 10 s with increasing GOx concentration from 1 U/mL to 50 U/mL. In contrast, the gelation time increased with increasing H_2O_2 concentration in the direct H_2O_2 addition process containing HRP from 10s (1 mM) to 60s (10 mM). A further increase in H_2O_2 concentration to 100 mM did not give hydrogelation within 30 min. The increase in gelation time with increasing concentration of directly added H_2O_2 has been explained by inactivation of HRP by excess H_2O_2. To evaluate the effects of the gradually supplied H_2O_2 on the cross-linking density of hydrogels, the mechanical properties of the GPT hydrogels were measure in time-controlled oscillatory mode with varying GOx and glucose concentration. Different with the case of H_2O_2-triggered system, the elastic modulus (*G'*) of hydrogel continues to increase depends on a time. In addition, higher GOx concentration shows an increase in the initial rate of hydrogelation. For instance, *G'* for a sample containing 100 U/mL reached 2000 Pa at 300 s, while *G'* for a sample containing 10 U/mL has reached 300 Pa. It has been shown that final mechanical properties of the hydrogels are directly affected by the H_2O_2 concentration for HRP-triggered hydrogelation system.[3] As expected, the final elastic modulus of the GPT hydrogels could be controlled by changing the glucose concentration (Figure 2). The *in vitro* cell viability result exhibited that the *in situ* forming GPT hydrogel *via* HRP-and GOx-mediated reaction is relatively non-toxic even at the highest concentration of 50 U/mL after 24 h culture, while direct H_2O_2 addition system significantly reduced cell viability up to 50%.

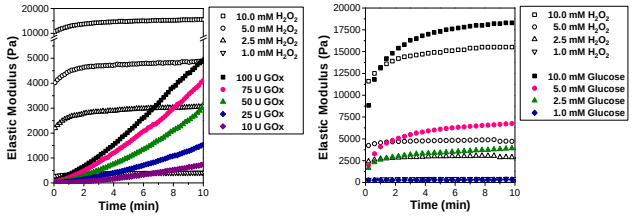

Figure 2. Time-course elastic modulus of GPT hydrogels with different concentration of GOx and glucose

Conclusions: The *in situ* cross-linkable GPT hydrogels are successfully prepared *via* HRP-and GOx-mediated reaction, and the physico-chemical properties of the hydrogel matrices could be controlled by varying material parameters. The obtained results support our hypothesis that GOx can catalyze an *in situ* hydrogelation of GPT conjugates in the presence glucose and HRP. Taken together, we expect that the dual enzyme-mediated *in situ* hydrogelation can be used as a certain alternative to the previous conventional that has been widely used to prepare the enzymatically cross-linkable hydrogels.

References:

[1] Teixeira LS, et al. Biomaterials. 2012;33:1281-1290.
[2] Kurisawa M, et al. Chem Comm. 2005;34;4312-4314.
[3] Park KM, et al. J Mater Chem. 2011;21;13180-13187.

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