

## Tissue adhesive gelatin hydrogels formed *in situ* via dual enzymatic crosslinking

Phuong Le Thi, Yunki Lee, Jin Woo Bae, Dai Hai Nguyen, Ki Dong Park\*

Department of Molecular Science and Technology, Ajou University, Suwon, Republic of Korea  
(\*kdp@ajou.ac.kr)

**Statement of Purpose:** Over the past decades, various types of *in situ* forming hydrogels have been developed for a wide range of biomedical applications. They can easily seal or fill damaged tissue, thereby functioning as cell/drug delivery vehicles or hemostats. In this regard, stable adhesion of materials to the surrounding tissue is considered important in improving *in vivo* performance of *in situ* forming hydrogels. Recently we have shown that phenol-derivatized gelatin could form a hydrogel *in situ* via horseradish peroxidase (HRP)-catalyzed crosslinking. They exhibited improved tissue adhesion through phenolic coupling between phenol-derivatized gelatin and tyrosine residues of tissue. However, a limited number of tyrosine residues on tissue surfaces might impede maximizing the extent of phenolic coupling. Tyrosinase is known as an oxidase that converts phenol into *o*-quinone.<sup>1</sup> The *o*-quinone form generated by tyrosinase can rapidly react with nucleophiles (e.g. amines or thiols). Accordingly, we hypothesized that use of tyrosinase may result in additional coupling between *o*-quinones of the derivatized gelatin and nucleophiles on tissue surfaces (Figure 1). This study presents a dual enzymatic crosslinking system based on HRP and tyrosinase to prepare gelatin hydrogels with improved tissue adhesion.

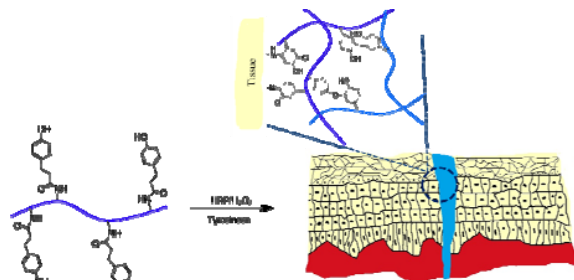


Figure 1. *In situ* forming GHPA hydrogel using dual enzymatic crosslinking reactions.

**Methods:** Gelatin-hydroxyphenyl propionic acid (GHPA) conjugate was synthesized and characterized as previously described.<sup>2</sup> Gelation time of GHPA was determined using a vial tilting method by varying concentrations of HRP (0.00125 – 0.01 mg/mL) and tyrosinase (0.1 – 5 kU/mL). Cohesive strength of GHPA hydrogels formed by HRP, tyrosinase or HRP-tyrosinase, was measured using a rheometer. For tissue adhesion test,

hydrogels were placed on cleaned porcine skins and then covered immediately with additional skins. Tissue adhesive strength of hydrogels was measured using a universal testing machine according to the modified ASTM method. Enzymatic degradation test was performed by incubating samples in PBS with different concentrations of collagenase. Cell viability was evaluated by 3D culture of human dermal fibroblasts (hDFB).

**Results:** The gelation time of GHPA varied from 5 s to 10 min, depending on the concentrations of HRP and tyrosinase. Two kinds of GHPA hydrogels formed by HRP-tyrosinase or HRP alone exhibited the highest cohesive strengths (Figure 2). On the contrary, tyrosinase itself could result in the lowest cohesive strength of the GHPA hydrogel though it initiated an enzymatic crosslinking reaction. The highest tissue adhesive strength of 34 kPa was obtained using HRP-tyrosinase, which was almost a 2-fold higher than that of the hydrogel prepared with HRP (19 kPa). Fibrin glue used as a control, which is commercially available, had the lowest tissue adhesion strength. All hydrogels were completely degraded in the presence of collagenase. Without collagenase, 60 wt% of hydrogel matrices remained stable over 2 days. After 3 days culture, the encapsulated hDFBs were almost viable and no dead cells were observed within the gel matrices, indicating excellent cyto-compatibility of GHPA hydrogels.

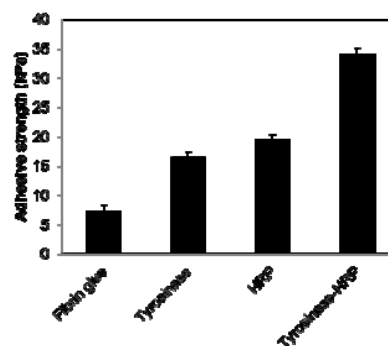


Figure 2. Adhesive strength of GHPA hydrogels formed *in situ* by different enzymatic cross-linking reactions.

**Conclusions:** A dual enzymatic crosslinking system based on HRP and tyrosinase was developed to prepare tissue adhesive GHPA hydrogels. The obtained results clearly demonstrated that tissue adhesion of GHPA hydrogels formed *in situ* by HRP-tyrosinase was significantly improved through additional coupling reactions between *o*-quinones of GHPA and nucleophiles on tissue surfaces. The improved tissue adhesion might be attributed to synergistic effects of HRP and tyrosinase that contribute to cohesive strength and adhesive

strength, respectively. Therefore, we expect that the dual enzymatic crosslinking system can be very useful for the development of tissue adhesive hydrogels.

**References:**

[1] Teixeira M, et al. *Biomaterials*. 2012;33:1281-1290.

[2] Lee Y, et al. *J Mater Chem B*. 2013;1:2407-2414.

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