Enhanced tissue adhesiveness of poly(ethylene glycol) based hydrogels *via* enzymatic crosslinking reaction

Simin Lee, Phuong Le Thi and Ki Dong Park*
Department of Molecular Science and Technology, Ajou University, Suwon, Republic of Korea
(*kdp@ajou.ac.kr)

Statement of Purpose: Injectable poly(ethylene glycol) (PEG) based hydrogels have been widely used for various biomedical applications because of their excellent biocompatibility and non-immunogenicity. However, the lack of ability to promote cellular behaviors (e.g., attachment, proliferation, migration) due to bioinert property of PEG have limited their applications for tissue repair and regeneration. Therefore, the blending of PEG with other natural polymers, such as gelatin, hyaluronic acid, chitosan,… has been exploited to improve both mechanical and biological properties of PEG based hydrogels. In this study, we developed two hybrid hydrogel systems with controllable tissue adhesiveness, by chemically crosslinking PEG with either gelatin or chitosan *via* horseradish peroxidase (HRP)-mediated reaction. The obvious advantages of our hydrogel systems are easy and large-scale preparation, by tuning the polymer composition and PEG architectures to obtain hydrogels with wide adjustability in mechanical strength.

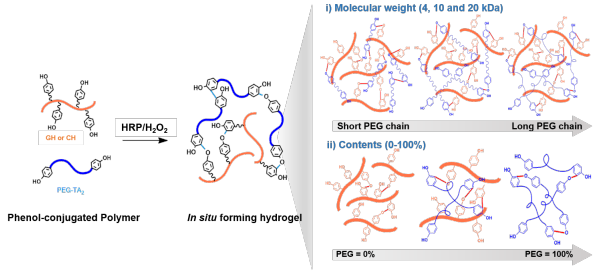

Figure 1. Schematic illustration for *in situ* formation of GH/PT and CH/PT hydrogels via HRP-mediated cross-linking reaction

Methods: Gelatin, chitosan and PEG with different molecular weight (4; 10 and 20 kDa) were functionalized with phenol moieties, termed as GH, CH, and PT, respectively. The hydrogel precursor solutions were prepared by simply mixing GH/CH and PT with different composition (10/0; 7/3; 5/5; 3/7 and 0/10 in wt/wt). Then, the hydrogels were rapidly form after adding HRP and H_2O_2 to the polymer solutions. The mechanical properties (compressive, tensile, and adhesive strength) of hydrogels were measured using a universal testing machine (UTM). The *in vitro* cell attachment study was carried out using human dermal fibroblasts (hDFBs). Furthermore, the hemostatic and wound healing capabilities of these adhesive hydrogels were evaluated using mice and rat models, compared to commercially available fibrin glue.

Results: The GH/PT and CH/PT hydrogels were rapidly formed with controllable gelation time from tens to hundreds sec by varying the polymer composition. The mechanical strengths of hybrid hydrogels, including compression, tensile and adhesion were significantly improved compared to the pristine PEG, gelatin and chitosan hydrogels. Notably, the hybrid hydrogels composed of 70% PT showed > 10 times greater tissue adhesiveness than commercially available fibrin glues. This result was explained by the enhanced mechanical strengths of the hybrid hydrogels. From *in vitro* cells studies, the hybrid hydrogels are nontoxic and improve cell proliferation, which is attributed to the bioactivity of natural polymers (gelatin and chitosan). Importantly, these hybrid hydrogels exhibited excellent hemostatic capability and accelerated the wound healing *in vivo*, compared to commercially available fibrin glue

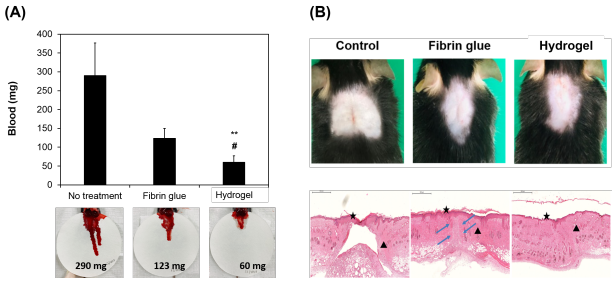

Figure 2. (A) *In vivo* hemostatic effect of CH/PT hydrogels evaluated by the total blood loss from a damaged mouse liver model. (B) *In vivo* wound closure effect GP hydrogels, compared to control (no treatment) and fibrin glue after 7 days of treatment

Conclusions: A simple enzymatic cross-linking method to prepare injectable PEG based hydrogel with high and controllable tissue adhesiveness has been developed. The mechanical as well as the adhesive strength of hydrogels was tunable by changing the M.W. and the content of PEG. The *in vivo* models demonstrated that hydrogels could rapidly crosslink *in situ* to stop bleeding and seal the wounds. These results suggested the potential of *in situ* cross-linkable hybrid gelatin/chitosan and PEG hydrogels as advanced bioadhesives for various biomedical applications.

References:

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