Preparation and characterization of *in situ* forming hybrid hydrogels composed of gelatin and poly(ethylene glycol)

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Statement of Purpose: *In situ* forming hydrogels have been extensively studied as versatile scaffolds for tissue engineering applications because they provide a convenient platform for local delivery of cells and/or drugs to target sites. For effective tissue regeneration using *in situ* forming hydrogels, various properties such as gelation time, mechanical strength and biodegradation rate need to be manipulated appropriately. Previously we have demonstrated two kinds of *in situ* forming gelatin hydrogels, gelatin-poly(ethylene glycol)-tyramine (GPT) and gelatin-hydroxyphenyl propionic acid (GHPA), which were prepared by a horseradish peroxidase (HRP)-catalyzed reaction. This HRP-catalyzed hydrogel system enabled fine control over crosslinking reactions of GPT and GHPA under mild conditions and thus resulted in tunable properties for both hydrogels. These hydrogels appeared promising to use as cell delivery vehicles. However, the GPT hydrogel with a relatively high content of PEG showed low initial cell attachment while the GHPA hydrogel was enzymatically degraded quickly due to a large portion of gelatin susceptible to enzymatic degradation. To overcome limitations of each hydrogel system, we prepared hybrid hydrogels composed of gelatin and PEG by varying the mixing ratio of GHPA and GPT. Gelation time, elastic modulus and degradation rate of GHPA/GPT hybrid hydrogels were investigated. Cell viability of the hydrogels was assessed using human dermal fibroblasts (hDFBs).

Methods: GPT and GHPA conjugates were synthesized and characterized as previously described in our papers.[1,2] Aqueous solutions of GHPA and GPT were mixed together in different ratios, followed by mixing with HRP and H_2O_2 to prepare GHPA/GPT hybrid hydrogels (Figure 1). Gelation time was investigated by a vial-tilting method. Swelling behaviors of the hybrid hydrogels was monitored at room temperature and 37 °C. Rheological analysis was performed to measure mechanical properties of the hybrid hydrogels. For proteolytic degradation study, 0.001 wt% of collagenase was used in aqueous media and changes in hydrogel weights were recorded. *In vitro* cell attachment and proliferation tests were carried out by 3D culture of hDFBs in hydrogels.

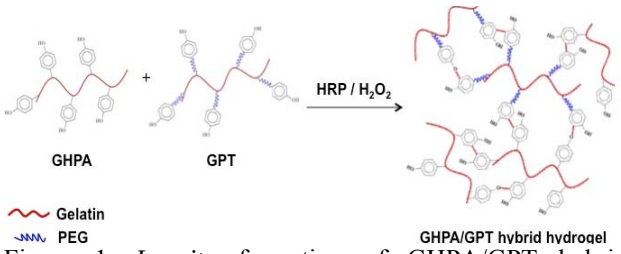

Figure 1. *In situ* formation of GHPA/GPT hybrid hydrogels via HRP-catalyzed crosslinking.

Results: By controlling the amounts of HPA and TA, the GHPA and GPT conjugates with the same phenol contents were prepared (150 μmol per 1g of polymer). The gelation time of GHPA/GPT hybrid hydrogels could be controlled easily by varying the HRP concentration, ranged from 5 s to 60 s. We found that increasing the ratio of GPT led to a significant decrease in the gelation time of the hybrid hydrogels. This faster gelation is most likely due to increased contents of flexible PEG chains. The elastic modulus of the hybrid hydrogels increased with increasing the GPT ratio, which might originate from a higher elasticity of PEG as compared to that of gelatin (Figure 2a). As expected, all hydrogels were degraded by collagenase. When the weight ratio of GPT increased from 0 to 100%, the degradation rates of hybrid hydrogels were prolonged from 5 to 37 days (Figure 2b), indicating that increasing the content of GPT could make hybrid hydrogels more durable. hDFBs cultured in hybrid hydrogel matrices appeared viable for 10 days.

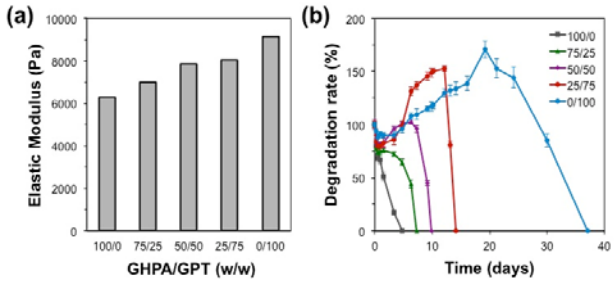

Figure 2. Elastic modulus changes and in vitro proteolytic degradation rate with varying GHPA/GPT ratios (w/w).

Conclusions: This study describes *in situ* forming GHPA/GPT hybrid hydrogels prepared by a HRP-catalyzed crosslinking reaction. By varying mixing ratios of GHPA and GPT, various properties of the hybrid hydrogels could be controlled. In particular, it was found that the total content of PEG in the hybrid hydrogel system affect significantly hydrogel properties. The obtained results demonstrated that our *in situ* forming gelatin-based hybrid hydrogels with tunable properties and good biocompatibility hold great promise as a versatile scaffold for tissue engineering applications.

References:

[1] Park KM, et al. J Mater Chem. 2011;21;13180-13187.
[2] Lee Y, et al. J Mater Chem B. 2013;18;2407-2414.

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