Enzyme-mediated injectable hydrogels composed of Tetronic and gelatin for tissue engineering

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Statement of Purpose: Injectable hydrogel systems are widely exploited as biomaterials for tissue engineering and drug delivery due to easy application based on minimally invasive technique. Recently, end-group-specific chemical reaction (e.g. michael-type addition and enzymatic reaction) are widely investigated. In particular, the enzyme-mediated cross-linking systems have various advantages such as biocompatibility and easy to control of reaction rate in mild condition.

In this study, enzyme mediated injectable hydrogels composed of Tetronic-tyramine (Tet-TA) and gelatin-hydroxyphenylacetic acid (GHPA) conjugates were developed as an injectable material for tissue engineering and drug delivery. The hydrogels were rapidly formed by using horseradish peroxidase (HRP) and H_2O_2. In this system, HRP catalyzes the coupling of phenol and aniline derivatives through the decomposition of hydrogen peroxide at the presence of aromatic proton donors. Their physico-chemical properties and *in vitro* cell and *in vivo* anmal study were investigated.

Methods: Tet-TA and GHPA conjugates were synthesized by a common carbodiimide/active ester-mediated coupling reaction. The chemical structure of the conjugates was characterized by ^{1}H NMR. Tet-TA/GHPA hybrid hydrogels were formed in the presence of HRP and H_2O_2 under physiological conditions as shown Figure 1. Their physico-chemical properties such as gelation time, degradation time, and mechanical properties were evaluated depending on the concentration of polymer ratio, HRP and H_2O_2 concentration.

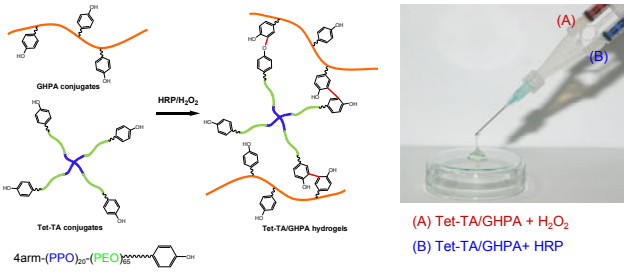

Figure 1. The schematic of enzyme-mediated hydrogel formation of Tet-TA/GHPA conjugates.

Results: The chemical structures were characterized by ^{1}H NMR spectrum, showing peaks at 6.91-7.23 ppm due to the presence of the tyramine (TA) substituent. The gelation time test was investigated by vial tiling method. The gel formation time decreases when increasing the HRP concentration at a constant polymer solution and H_2O_2 concentration. This result may be due to the increased rate of creating phenoxy radicals. Increasing H_2O_2 concentration at a constant polymer solution and HRP concentration, on the other hand, resulted in increases of the gelation time. This may be due to excessive oxidation of HRP by H_2O_2. The gelation time and mechanical properties were controlled by the variation of polymer ratio (Tet-TA:GHPA), HRP, and H_2O_2 concentration. In addition, *in vitro* cell study using osteoblast was investigated on the hydrogels. The cultured cells were observed by fluorescence staining. *In vitro* cell study demonstrated that the cells remained viable and were proliferated for 3 days culture period. The cytoskeleton of the cultured cells observed by fluorescent staining showed that the F-actin stress fibers of the cells were appreciably spread on the hydrogels after 3 days as shown Figure 2.

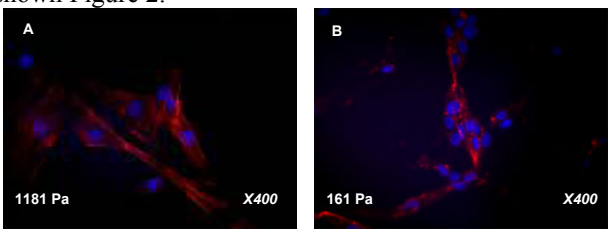

Figure 2. F-actin assay images of osteoblast on the Tet-TA/GHPA hydrogel matrix after 3 days depending on the ratio of Tet-TA:GHPA conjugates (A) 7:3 and (B) 5:5.

Furthermore, *in vivo* subcutaneous injection was carried out using white rabbit. The Tet-TA/GHPA hydrogels were injected simply using double syringe as shown Figure 3. The histological analysis is on going.

Figure 3. Subcutaneous injection using double syringe.

Conclusions: Enzyme mediated injectable hydrogels composed of Tet-TA and GHPA conjugates were developed. The obtained results demonstrated that the Tet-TA/GHPA hydrogel is a promising injectable material for tissue engineering and drug delivery.

References:

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