Non-invasive *in vivo* evaluation of 3-D cell proliferation in the *in situ* cross-linkable gelatin hydrogels with different matrix stiffness

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Statement of Purpose: *In situ* cross-linkable hydrogels are widely exploited as biomaterials for tissue regeneration and drug delivery due to easy application based on minimally invasive technique. Recently, the injectable hydrogels have been used in regenerative medicine to provide biomechanical support for stem cells and to fill up lost tissue area together with stem cells.

In this study, *in situ* forming gelatin-based hydrogels via enzyme-mediated reaction using horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) was developed as an injectable material for tissue regenerative medicine. The enzyme-mediated cross-linking systems have various advantages such as biocompatibility and easy to control of the reaction rate in mild condition. The hydrogels were rapidly formed by using HRP and H_2O_2. Their physico-chemical properties were characterized and the proliferation of mouse neuronal stem cells (mNSC) within the hydrogel formed with different mechanical properties was evaluated via *in vitro/in vivo* bioluminescence monitoring.

Methods: The gelatin derivative was synthesized by a common carbodiimide/active ester-mediated coupling reaction. The chemical structure of the derivatives was characterized by ^{1}H-NMR. The gelatin based hydrogels were formed in the presence of HRP and H_2O_2 under physiological conditions as shown in Figure 1. Their physico-chemical properties such as gelation time, degradation rate, and mechanical properties were evaluated depending on the concentration of catalysts. *In vitro* cell study was investigated using stable cell line transduced with an enhanced firefly luciferase retroviral vector (mNSC-effLuc) within the hydrogel formed with the different stiffness. For *in vivo* study, the hydrogels with mNSC-effLuc were subcutaneously injected onto the back of mouse. The luciferase activity was monitored by bioluminescence imaging.

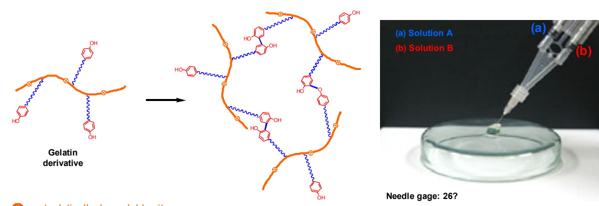

Figure 1. The schematic route of enzyme-mediated hydrogel formation.

Results: The chemical structure was 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 tilting method. The gelation time decreased 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 peroxidase by H_2O_2. The gelation time and mechanical properties could be controlled by the variation of the catalysts. The gelation time ranged from 5 sec to 2 min approximately and the mechanical strength ranged from 200 Pa to 8000 Pa (3 wt% of hydrogel).

In addition, *in vivo/in vitro* study was investigated using mNSC-effLuc within the hydrogels formed with different mechanical strength. The cultured cells were monitored by bioluminescence imaging. The bioluminescence intensity increased when the matrix stiffness decreased, which can be explained by the fact that cells in the softer matrix were well proliferated than those in the stiffer matrix. Figure 2 shows the images of *in vivo/in vitro* bioluminescence image.

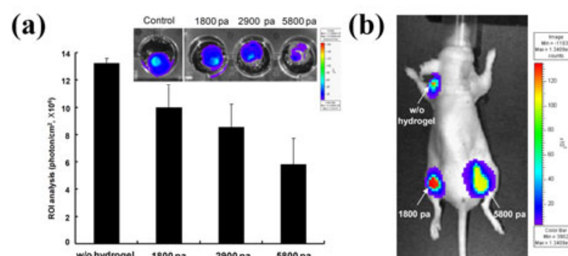

Figure 2. The bioluminescence image of mNSC-effLuc cultured within the hydrogels depending on the matrix stiffness: (a) *in vitro* and (b) *in vivo*.

Conclusions: *In situ* forming gelatin-based hydrogel was developed and the proliferation of mNSC-effLuc incorporated in the hydrogel matrices with different matrix stiffness was monitoring via *in vitro/in vivo* bioluminescence imaging. Obtained results demonstrated that the non-invasive monitoring system using the *in situ* forming gelatin based hydrogels may be a powerful tool to evaluate the characteristics of stem cells within the matrix for therapeutic application in tissue regenerative medicine.

References:

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