In situ forming gelatin-based hydrogels via enzyme-mediated reaction for tissue regeneration and drug delivery

K. M. Park, Y. K. Joung, K. D. Park*
Department of Molecular Science and Technology, Ajou University, Suwon, Republic of Korea
(kdp@ajou.ac.kr)

Statement of Purpose: *In situ* forming hydrogels, often referred to as injectable hydrogels, are widely exploited as biomaterials for tissue regeneration 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 the reaction rate in mild condition. Gelatin is commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility in physiological environments.

In this study, *in situ* forming gelatin based hydrogels via enzyme-mediated reaction was developed as an injectable material for tissue regeneration and drug delivery. The hydrogels were rapidly formed by using peroxidase and hydrogen peroxide. Their physico-chemical properties and *in vitro* cell study were investigated.

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 peroxidase and hydrogen peroxide under physiological conditions as shown in Figure 1. Their physico-chemical properties such as gelation time, degradation time, and mechanical properties were evaluated depending on the concentration of peroxidase and hydrogen peroxide.

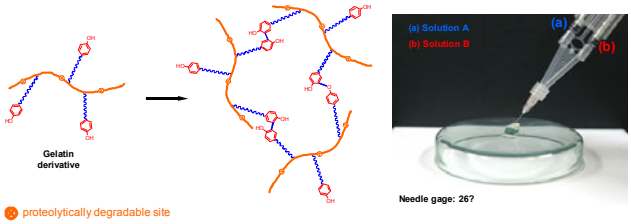

Figure 1. The schematic route of enzyme-mediated hydrogel formation.

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 peroxidase concentration at a constant polymer solution and hydrogen peroxide concentration. This result may be due to the increased rate of creating phenoxy radicals. Increasing hydrogen peroxide concentration at a constant polymer solution and peroxidase concentration, on the other hand, resulted in increases of the gelation time. This may be due to excessive oxidation of peroxidase by hydrogen peroxide. The gelation time and mechanical properties could be controlled by the variation of the peroxidase and hydrogen peroxide. The gelation time ranged from 5 sec to 2 min approximately and the mechanical strength ranged from 80 Pa to 6000 Pa. In addition, *in vitro* study was investigated using various cell types on the hydrogel matrices. The cultured cell was observed by phase contrast. The cells were well attached and proliferated on the hydrogel matrices. Figure 2 shows the images of the cultured cells on the hydrogel matrices for 24 hr.

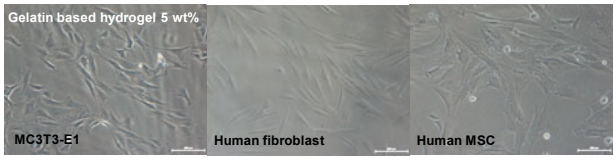

Figure 2. The phase contrast images of the cultured cells on the hydrogel matrices for 24 hrs.

Furthermore, *in vitro* 3-demensional cell culture in the hydrogel matrices was carried out using rat MSC for 5 days. Cells were stained with fluorescent dyes (calcein AM and ethidium homodimer-1). Living cells stained with calcein AM fluoresce green, while dead cells fluoresce red. Spreading and elongation of cells through hydrogel matrices was observed as shown in Figure 3. Fibrin gel was used as a control group.

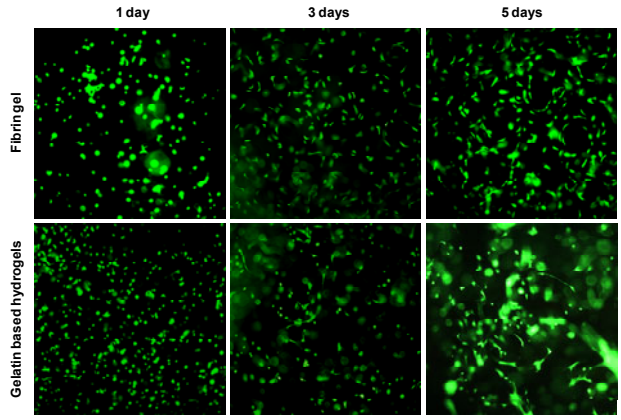

Figure 3. Fluorescence microscopy images of rat MSC in the hydrogel matrix (3D cell culture).

Conclusions: *In situ* forming gelatin-based hydrogel was developed as an injectable scaffold. Obtained results demonstrated that enzyme-mediated gelatin hydrogel is a promising injectable material for tissue regeneration and drug delivery.

References:

1) M. Kurisawa, J. E. Chung, Y. Y. Tang, S. J. Gao, H. Uyama, Chem. Commun. 4312, 2005
2) R. Jin, C. Hiemstra, Z. Zang, J. Feijen, Biomaterials, 28, 2791, 2007.

Acknowledgements: This work was supported by grants from The National Research Foundation of Korea (NRF) grant funded by the Korea government (20090085779) and Korea Health 21 R&D Project, Ministry of Health Welfare & Family Affairs, Republic of Korea (A091120).