Stimulus-Responsive Degradation of Biocompatible Polypeptide Hydrogel for Tissue Engineering

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Statement of Purpose: In tissue engineering, the three-dimensional (3-D) cell culture within the biodegradable hydrogels such as poly($_L$-lactic acid) and collagen have been performed actively to create a 3-D tissue-like structure. However the development of 3-D tissue-like structure is difficult because of the blocking of diffusion of culture medium in the late stage of cell culture by the dense fibril structure of hydrogels. Furthermore, the fabrication of micrometer size blood vessels in 3-D tissue-like structure is required. To solve this problem, we designed the stimulus-responsive biocompatible polypeptide hydrogel crosslinked by disulfide bond. It is known that disulfide bond is cleaved by reducing reagents such as dithiothreitol (DTT) and glutathione (GSH)[1]. If disulfide-crosslinked polypeptide hydrogel can be dissolved in the late stage of 3-D cell culture within hydrogel, 3-D tissue-like cell construct having the micrometer size pores corresponding to the fibril structure of hydrogel may be created. The 3-D tissue-like cell construct will be cultured for a long term in vitro, because the blocking of diffusion of culture medium will not occur by the porous structure. The advantage of this method is the development of 3-D cell construct composed of cells and extracellular matrix only because of the complete dissolution of hydrogel. Furthermore, the fabrication of micrometer size blood vessels inside of the cell construct is expected by the co-culture with vascular endothelial cells.

Here, we report the preparation of stimulus-responsive polypeptide hydrogel crosslinked by disulfide bond. Furthermore, the controlling of degradability and the cell adhesion property of the hydrogel were evaluated.

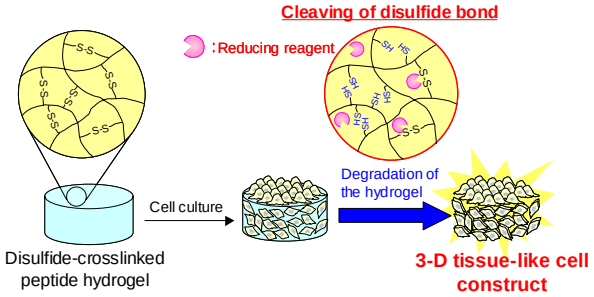

Figure 1. Schematic illustration of 3-D tissue-like cell construct.

Methods: [Preparation of disulfide-crosslinked poly(γ-glutamic acid) hydrogels]

5 unit mmol of poly(γ-glutamic acid) (γ-PGA) and 5 mmol of water soluble carbodiimide were dissolved in 10 mL of 0.5 M sodium hydrogen carbonate solution under magnetic stirring for 15 min at 4 °C. 2.5 mmol of cystamine or cystine as a disulfide crosslinker was added to the solution. The reaction solution was poured into the glass plates with 1 mm silicon rubber, and it was maintained for 3 hours. The remaining compounds in the hydrogels were removed by soaking in pure water for 3 days.

[Degradability test of hydrogels with reducing reagents]

The cystamine- or cystine-crosslinked γ-PGA hydrogels were immersed into 50 mL of distilled water, phosphate buffered saline (PBS), and Eagle's MEM (10 % FBS) with or without DTT or GSH. The degradation time of the hydrogels was observed by visual evaluation.

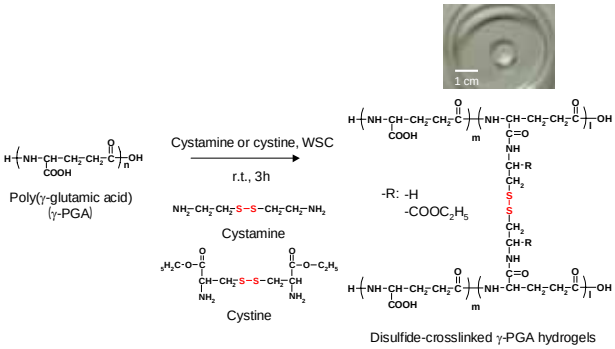

Figure 2. Preparation of disulfide-crosslinked γ-PGA hydrogels.

Results / Discussion: Cystamine- or cystine-crosslinked γ-PGA hydrogels were successfully prepared (Figure 1). The degradability test of the γ-PGA hydrogels indicated that all of hydrogels were stable in water, PBS, and EMEM without reducing reagents for 7 days at 37 °C. When DTT or GSH were added to the solutions, almost of γ-PGA hydrogels were gradually degraded. The degradation time of the hydrogels were controllable from 0.5 h to 1 week by controlling of crosslinkers, solutions, reducing reagents, and swelling ratio of the hydrogels. We successfully prepared L929 fibroblast cell construct after the degradation of hydrogels by GSH (Figure 3 (B)).

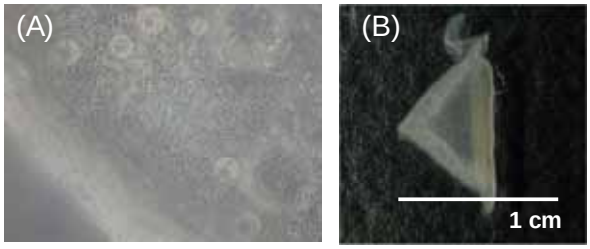

Figure 3. L929 fibroblast cell culture onto the cystamine-crosslinked γ-PGA hydrogel for 7 days (A), and L929 cell construct after the degradation of the hydrogel by 1.0 mM of GSH (B).

Conclusions: We successfully prepared disulfide-crosslinked polypeptide hydrogels and controlled the degradability of the hydrogels. Our hydrogel system will be useful for in vitro tissue engineering.

References: 1) N. Hisano, N. Morikawa, H. Iwata, Y. Ikada. *J. Biomed. Mater. Res.*, **40**, 115-123 (1998).