Hyaluronic Acid-Catechol Hydrogel for Liver Tissue Engineering

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Statement of Purpose: Orthotopic liver transplantation is the only possible treatment for the patients with end-stage liver diseases. However, due to the shortage of donor liver for transplantation, it is desirable to develop alternative therapies for liver regeneration. Liver tissue engineering has been considered as one of the alternative strategies for liver tissue regeneration and restoration of functions of a largely damaged liver. However, it is crucial to maintain viability and functions of transplanted hepatocytes *in vivo* for a period of time because primary hepatocytes easily lose their phenotype, viability, and functionality. In this study, we developed a mussel-inspired hyaluronic acid (HA) hydrogel as a novel tissue engineering scaffold for efficient hepatocyte transplantation. A mitogenic growth factor (basic fibroblast growth factor; bFGF), which may support viability and functions of encapsulated hepatocytes, was also incorporated into the hydrogel. The viability and hepatic functions of hepatocytes in the hydrogel were examined both *in vitro* and *in vivo*.

Methods: HA-catechol was synthesized by conjugating dopamine to HA backbones. HA-catechol conjugate was crosslinked by adding an oxidation agent in a basic condition to construct hydrogels. Two types of hepatocytes were encapsulated into the HA-catechol hydrogels, including primary mouse hepatocytes isolated by collagenase perfusion method and human hepatocytes. Hepatocyte encapsulation group with bFGF supplementation was compared to the group of encapsulating cells alone in terms of viability and hepatic functions. The viability of encapsulated hepatocytes was determined by live/dead staining at several time points during the culture. For testing *in vitro* functions, the level of albumin and urea production from hepatocytes were quantified using albumin ELISA kit and urea-nitrogen direct test kit, respectively. The gene expressions of hepatocyte specific markers (albumin, CYP7A1, HNF4A, and G6PC3) were assessed with quantitative real-time polymerase chain reaction (qRT-PCR). Finally, human hepatocytes were transplanted by using HA-catechol hydrogels into the subcutaneous spaces and liver tissues of athymic mice. The hydrogel constructs with cells were retrieved two weeks post-transplantation and analyzed with histology and immunohistochemistry.

Results: HA-catechol conjugate was synthesized by a standard carbodiimide coupling chemistry. During the culture in the HA-catechol hydrogel, primary mouse hepatocyte encapsulated with bFGF showed viability two-fold higher than control group without bFGF supplementation (hepatocyte alone) (Figure 1). The urea synthesis and the gene expression of hepatocyte markers were significantly enhanced in the primary mouse hepatocytes encapsulated with bFGF in the hydrogels, indicating that HA-catechol hydrogel with bFGF supported phenotypic maintenance and functionality of hepatocytes *in vitro* 3D culture. In the 3D culture of human hepatocytes in the HA-catechol hydrogels, more than 80% of encapsulated human hepatocytes were found to be alive for two weeks. Human hepatocytes encapsulated with bFGF exhibited higher ability in urea and albumin synthesis than control group without bFGF (human hepatocytes alone).

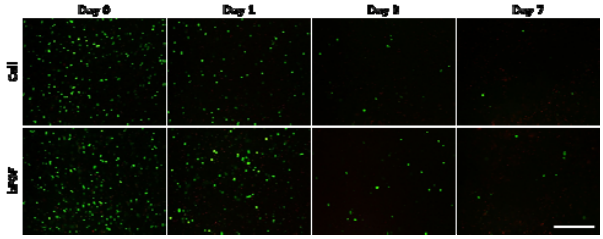

Figure 1. *In vitro* viability of primary mouse hepatocytes cultured in the HA-catechol hydrogels, which was determined by live/dead staining at days 0, 1, 3 and 7.

Finally, when human hepatocytes were transplanted into subcutaneous space of mice by using HA-catechol hydrogel, they were successfully engrafted *in vivo*. Upon transplantation to the liver, HA-catechol hydrogels were tightly attached onto the surfaces of the liver up to two weeks probably due to the presence of catechol moieties (Figure 2).

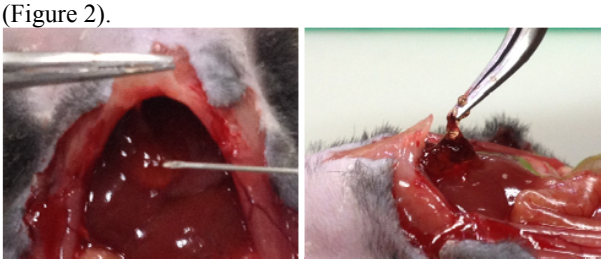

Figure 2. HA-catechol hydrogels attached onto the livers in mice.

Conclusions: Here we present a mussel-inspired, catechol-modified HA hydrogel for liver tissue engineering. The adhesive property of this hydrogel originated from catechols may be able to facilitate efficient engraftment of transplanted hepatocytes onto the liver tissues. Addition of bFGF into the hydrogel system is expected to further enhance viability and hepatic functions of transplanted hepatocytes *in vivo*.

References:

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