Heparin-Based Hydrogel as a Matrix for Cultivation of Primary Hepatocytes

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Statement of Purpose: Primary hepatocytes, a main cell type in liver, carry out most of the specialized functions of liver, including production of majority of the liver-specific proteins, regulation of the carbohydrate, urea, and lipid metabolism as well as detoxification of exogenous chemicals.[i] However, since primary hepatocytes rapidly lose their functions and viability after isolation, emphasis is focused on the maintenance of their function for a sufficiently long-term in *in vitro* primary hepatocyte culture. Formation of primary hepatocytes in 3-D is one of the approaches for a long-term culture, and the use of hydrogels is a promising way because of their high water content, and easy tuning of their modulus to that of the native liver. Previously, we developed an in situ forming heparin-based hydrogel that can be gelled in the presence of biomolecules.[ii] Heparin is capable of interacting with numerous ECM proteins and growth factors that have heparin-binding domain. Particularly, heparin is one of the main polysaccharides in liver,[iii] and it has a high affinity for hepatocyte growth factor (HGF), well known as a potent factor in growth and function of hepatocytes.[iv] In this study, we applied the heparin-based hydrogel with/without HGF for long term 3-D hepatocyte culture.

Methods: Heparin-based hydrogels were prepared by a Michael-type addition reaction between thiolated heparin and diacrylated PEG. HGF-contained hydrogels were prepared by adding HGF solution to gel precursor solution during gelation, resulting in 1 μg/ml final concentration of HGF. The HGF-release from heparin-based hodrogel was assayed by ELISA. Primary hepatocytes were isolated from adult female Lewis rats using a two-step collagenase perfusion and then cultured in the heparin-based hydrogels with/without HGF. For analysis of hepatic functions such as albumin and urea production, culture medium was collected every day and was analyzed using albumin and urea ELISA. PEG hydrogel was used as a control.

Results: The initial viability of hepatocytes after encapsulation was sufficiently high (over 70 %), (**Fig 1**) and the maintenance of their viability as well as functions over several weeks were observed. HGF in heparin hydrogel showed a much slower release profile. Over the course of three weeks hepatocyte spheroids entrapped in heparin hydrogels exhibited high levels of albumin (**Fig 2**) and urea synthesis with hepatic function increasing over time. Importantly, inclusion of HGF into the hydrogel reversed the loss of function in single hepatocytes and enhanced hepatic phenotype of hepatocyte spheroids. In contrast, the function of hepatocyte spheroids in PEG hydrogel was minimal, and the addition of HGF did not improve their hepatic function.

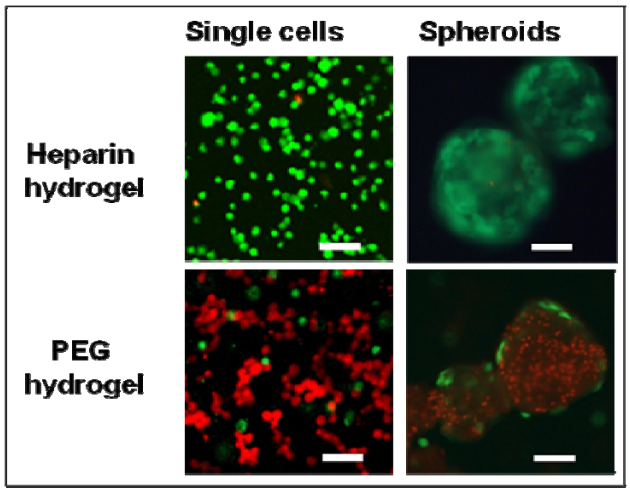

Figure 1. Cell viability at day 1 after encapsulation. Live/dead fluorescent staining images of hepatocytes in the form of single cells and spheroids in the both hydrogels. All scale bars are 200 μm.

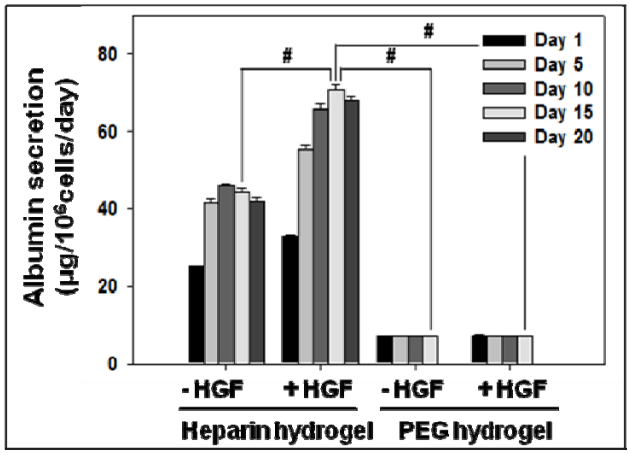

Figure 2. The normalized albumin production of encapsulated primary hepatocytes in the form of spheroids with or without the addition of HGF. (#) $p<0.0001$ (n=4).

Conclusions: Heparin hydrogel was found to be an excellent biomaterial for maintenance of functional primary hepatocytes, thus may be applicable as a matrix for differentiation of hepatocytes or stem cells *in vitro* and as a scaffold for transplantation of these cells.

References

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