New blood compatible polymers for the maintenance of hepatocyte functions to develop bioartificial liver.

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Statement of Purpose: Only treatment for severe liver diseases is liver transplantation although the number of donor is strictly limited. Due to this problem of donor limitation, it is expected to develop bioartificial liver (BAL) with hepatocytes. The substrate for bioartificial liver requires blood compatibility otherwise clot forms in BAL connected to blood circulation. However, classical blood compatible materials such as polyethylene glycol and poly (2-methacryloyloxyethyl phosphorylcholine) (MPC) do not allow hepatocyte attachment and hepatocytes undergo cell death on these materials. Moreover, primary hepatocytes lose their specific functions during the culture *in vitro*. Therefore, the substrate for BAL requires many criteria as follows: 1) the ability of hepatocyte attachment, 2) blood compatibility, and 3) the ability to maintain hepatocyte specific functions.

We have previously reported that blood compatible poly (2-methoxyethyl acrylate) (PMEA) allow cell attachment [1, 2]. Cells attach on PMEA via both integrin-dependent and –independent manners and the cells on PMEA exhibit round shape due to insufficient integrin signaling. It has been reported that hepatocytes maintain their specific functions when the cells form round shape [3]. Therefore, it is expected that hepatocytes maintain their specific functions on blood compatible PMEA by forming their shape to round and is also expected that PMEA can be used as the substrate for BAL. In this study, we used human hepatocarcinoma cell line, HepG2, as a model of human hepatocyte and examined HepG2 attachment, shape, and liver specific functions on PMEA and its analogous polymer, poly (tetrahydrofurfuryl acrylate) (PTHFA).

Methods: PMEA and PTHFA were spin-coated on polyethylene terephthalate (PET) discs. Also, poly (2-hydroxyethyl methacrylate) (PHEMA) and poly (2-methacryloyloxyethyl phosphorylcholine-*co*-butyl methacrylate) (PMPC) were also spin-coated on PET discs. Bare PET discs were immersed in 10 μg/ml of fibronectin (FN) solution to prepare FN-coated PET. To evaluate cell attachment on the substrates, HepG2 were seeded on prepared substrates at a density of 5×10^4 cells/cm^2 and were incubated for 3 h in 10% FBS containing DMEM/F-12 medium with or without 5 mM EDTA. Non-attached HepG2 cells were removed by washing with PBS twice. Attached cells were visualized by crystal violet staining to count. Also, the cells were visualized by crystal violet staining to measure projected cell areas with Photoshop and imageJ after 1 day culture. The expression levels of *HNF4A* and *ALB* were measured as indicator of liver specific functions by real-time PCR. F-actin and vinculin as a marker of focal adhesion were observed by fluorescent immunocytochemistry.

Results: HepG2 cells can attached on blood compatible PET, PMEA, and PTHFA although HepG2 hardly attached on FN, PHEMA, and PMPC without 3 h. Projected cell areas of HepG2 on PET, PTHFA, and FN were higher than those on PMEA and PHEMA, indicating that HepG2 kept round shape on PMEA compared with PET, PTHFA, and FN. *HNF4A* and *ALB* expression were evaluated as indicators of liver specific functions. The expression levels of *HNF4A* and *ALB* increased on PMEA compared with PTHFA and tissue culture polystyrene, suggesting that HepG2 exhibited higher liver specific functions on PMEA (Fig. 1).

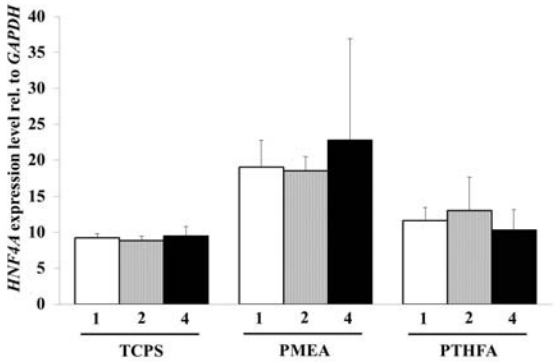

Fig. 1: *HNF4A* expression levels in HepG2 cultured on TCPS, PMEA, and PTHFA. Data represent mean±SD (n=3).

To evaluate attachment mechanism on PMEA and PTHFA, HepG2 attachment was evaluated in the presence of EDTA, an inhibitor of integrin attachment. HepG2 attachment was completely inhibited on PET, PTHFA, PHEMA, and FN whereas the attachment was partially inhibited on PMEA by EDTA, suggesting that HepG2 attached on PMEA via both integrin-dependent and –independent manners. To further confirm the contribution of integrin for HepG2 attachment, focal adhesions were observed after 1 day culture. Evident focal adhesions were observed on PET, PTHFA, and FN whereas few focal adhesions were observed on PMEA and PHEMA, suggesting that the contribution of integrin for cell attachment was weak and integrin signal was suppressed on PMEA. It has been well reported that integrin signaling promotes cell spreading. Therefore, HepG2 kept round shape on PMEA by the suppression of integrin signaling, leading higher expression of liver specific functions.

Conclusions: Blood compatible PMEA is expected to be used as a substrate for BAL development.

References:

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