Assembly of Functional Neovessels using a Stereolithographic Hydrogel Matrix

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Statement of Purpose: Hydrogels are increasingly being used as cell encapsulation devices for both fundamental biology studies and cell transplantation therapies because of their structural similarity to the natural extracellular matrix. The goal of this study is to develop an advanced cell-encapsulating hydrogel which can support cell viability and function to recreate vascular networks using tissue engineering technology. Here, we present a vascularized hydrogel matrix which is assembled by encapsulating cells into a hydrogel which presents desired level of stiffness and bulk permeability and also displays micro-sized channels using the stereolithogrphic apparatus (SLA). In this study, fibroblasts were encapsulated into the vascularized 3D hydrogel by the SLA based in situ photo cross-linking reaction. The role of micro-sized channels by combining a hydrogel formulation in enhancing cell-viability and regulating cell-functions to assembly functional neovessels was investigated.

Methods: A vascularized hydrogel was assembled by photo-crosslinking process with a stereolithography apparatus (SLA, Model 250/50, 3D systems) [3]. Gel-forming polymers, poly(ethylene glycol) diacrylates (PEGDA) and methacrylic alginate (MA)[1-2], were synthesized and used. In this study, the number of methacrylates linked to a single alginate with molecular weight (M_w) of 50,000 g/mol was kept constant at 60. The pre-gelled solution was made by mixing 2.0 mL of the PEGDA solution with varied amounts of MA while keeping the total gel-forming polymer concentrations constant.

The hydrogel stiffness was evaluated with measurement of the elastic modulus of a hydrogel. Following the incubation in the medium for 24 hours, the gel structure was compressed at a rate of 1.0 mm/min using a mechanical testing system (MTS Insight). The elastic modulus was calculated from the slope of a stress (σ) vs. strain (λ) curve at the first 10% strain. The hydrogel swelling ratio at equilibrium was determined by measuring the weight of the hydrated gel after 24 hours in neutral buffered solution at 37°C and that of the dried gel. The viability of encapsulated cell (NIH 3T3, ATCC) was evaluated by adding 0.1 mL of a growth medium and 0.01 mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) reagent (ATCC) into a well of a 96-well plate which contains each gel structure. The role of micro-sized channels by combining a hydrogel formulation was further examined by implanting the cell-hydrogel construct onto chicken chorioallontoic membrane (CAM).

Results: The hydrogel consisted of PEGDA and MA, so the hydrogel pore diameter was increased with mass fraction of MA while increasing elastic modulus in an independent manner [2]. In this study, fibroblasts were encapsulated into the vascularized 3D hydrogel by the SLA based in situ photo cross-linking reaction. We found that the fraction of viable cells was significantly increased with mass fraction of MA due to the increase of gel diameter in the nano-sized pore. The cell viability was further increased with micro-sized channels introduced into a hydrogel by the SLA. Further, the roles of incorporation of MA and micro-sized channels in regulating fibroblasts function to secret proangiogenic growth factors and subsequently promote the neovascularization in a connective tissue was evaluated using chorioallantoic membrane (CAM). Implantation of PEGDA hydrogel onto CAM stimulated inflammation within two days likely because of extravasation of dead cells' debris. In contrast, the PEGDA/MA hydrogel minimally stimulated host inflammation likely because of its ability to let the encapsulated cells remain viable. Interestingly, fibroblast-encapsulated PEGDA/MA containing microchannels increased the density of mature capillaries which present smooth muscle layers.

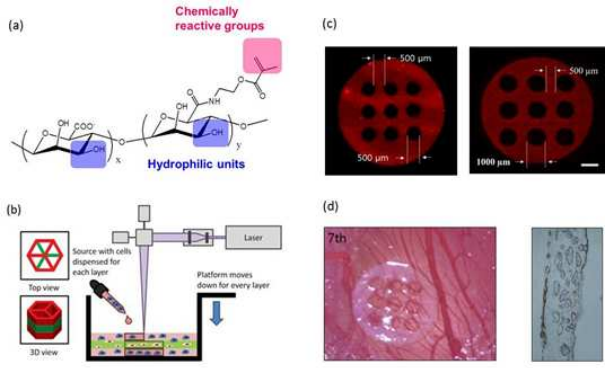

Figure 1. Schematic diagram of methacrylic alginate (MA) (a) and stereolithography (SLA) (b). (c) Stereolithographic hydrogel matrix with microchannels. (d) The cell-encapsulated hydrogels implanted onto chick embryo chorioallantoic membrane (CAM).

Conclusions: Taken together, these results demonstrate that the vascularized hydrogel matrix assembled by independently controlling mechanics and transport properties of a cell-encapsulated hydrogel provides enhancing cell-viability and regulating cell-functions to assembly functional neovessels. The results of this study will be an invaluable paradigm of a 3D cell encapsulation device prepared with a broad array of gel-forming polymers.

References: (1) Cha C. *Adv. Funct. Mater.* 2009; 19: 3056-3062, (2) H.J. Kong. *Biomaterials* 2010; 31: 4864-4871, (3) V. Chan, *Lab on a Chip* 2010; 10: 2062-2070.