DYNAMIC THREE-DIMENSIONAL MICROPATTERNED COCULTURE USING PHOTOCURABLE AND CHEMICALLY DEGRADABLE HYDROGELS FOR STEM CELL DIFFERENTIATION

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ABSTRACT

We report the spatiotemporal control of a 3D coculture by combining photocurable and chemically degradable hydrogels for coculture of embryonic stem (ES) cells with HepG2 cells. ES cells were encapsulated in micropatterned photocurable poly(ethylene glycol) (PEG) hydrogels. The cell-laden PEG hydrogels were then encapsulated in calcium-alginate (Ca-Alg) hydrogel containing HepG2 cells. The ES cells in the PEG hydrogels were then cocultured with the HepG2 cells in the Ca-Alg hydrogel for 4 days. The Ca-Alg hydrogel containing HepG2 cells was degraded by exposure to sodium citrate solution and the HepG2 cells were removed. ES-derived cells were then cultured in differentiation media.

KEYWORDS

Embryonic stem cells, Cardiac differentiation, Micropatterning, Coculture, Hydrogel.

INTRODUCTION

Conventional cell culture technique utilizes two dimensional (2D) monolayer culture system with spatially uniform and static environments. This 2D culture system lacks the heterogeneity and dynamics that exist *in vivo*. Recently, many research groups have reported the construction of 3D microstructures of hydrogels by means of a top-down approach using photocurable materials [1-5]. Others have used a bottom-up approach to construct 3D microenvironments using cell assembly and hydrogels [6-9]. Furthermore, numerous methods have been developed to dynamically control intracellular interactions and cell migration on two-dimensional (2D) surfaces. However, none of these techniques can be applied to dynamically control the 3D microenvironment.

Anseth et al. reported that photodegradable hydrogels composed of poly(ethylene glycol)-di-photodegradable acrylate (PEGdiPDA) could be used for spatiotemporal control of 3D microstructures [10]. They encapsulated cells to maintain their viability and successfully demonstrated cellular responses to the dynamic environment. However, their synthetic material is not commercially available and the equipment they used is not available in most biology laboratories.

Here, we report the spatiotemporal control of a 3D coculture environment by combining photocurable and chemically degradable hydrogels. This chemically degradable hydrogel is more biocompatible and convenient, and less expensive, for use in 3D cocultures compared with the synthetic photodegradable crosslinker, PEGdiPDA [10]. We applied our dynamic 3D micropatterned coculture to the coculture of mouse embryonic stem (ES) cells with HepG2 cells, the conditioned media of which induces early-stage differentiation of ES cells [11].

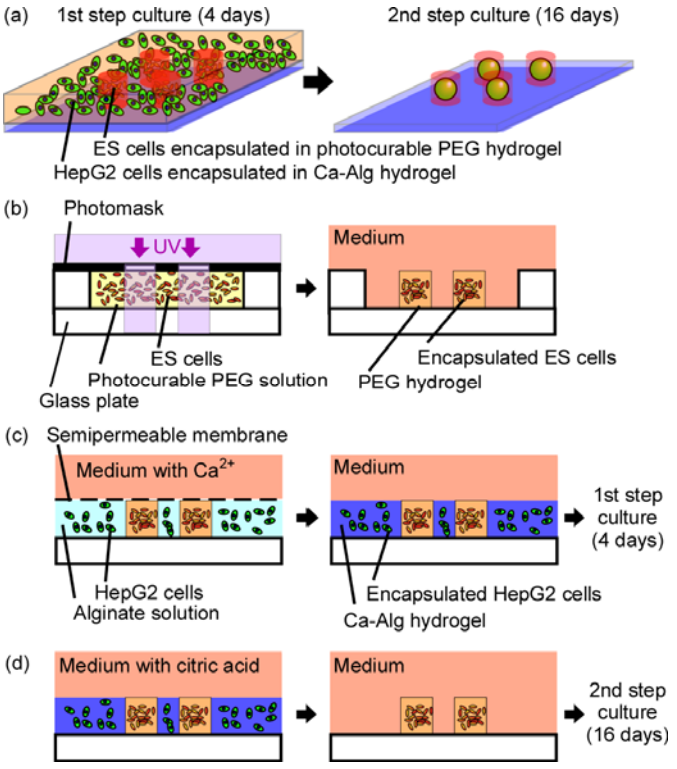

Figure 1: Schematic of the dynamic 3D micropatterned coculture. (a) In the 1st step culture, ES cells in photo-curable-PEG are cultured with HepG2 cells in a Ca-Alg hydrogel for 4 days. In the 2nd step culture, the ES cells are cultured without the HepG2 cells, which are removed after the Ca-Alg hydrogel is degraded for 16 days. (b) Encapsulation of ES cells in the photocurable PEG hydrogel. (c) Encapsulation of HepG2 cells in the Ca-Alg hydrogel. (d) Degradation of the Ca-Alg hydrogel and removal of HepG2 cells after the 1st step culture for 4 days.

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EXPERIMENT

Dynamic 3D micropatterned coculture

Fig. 1 shows the dynamic 3D micropatterned coculture. Mouse ES cells were encapsulated in the micropatterned photocurable PEG hydrogels (Fig. 1b). Calcium-alginate (Ca-Alg) hydrogel containing HepG2 cells was formed around the cell-laden PEG hydrogels (Fig. 1c). These cells were cocultivated under 3D coculture conditions for 4 days in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. The Ca-Alg hydrogel was then degraded by exposure to 50 mM sodium citrate solution in DMEM for 10 min (Fig. 1d), which allowed the 3D coculture environment around the ES cells to be dynamically controlled. After degradation of the Ca-Alg, the ES cells were cultivated without HepG2 cells for 16 days in α-Minimum Essential Medium containing 15% ES cell qualified-FBS to induce cardiac differentiation.

Evaluation of cell micropatterning and viability

To visualize the cells during dynamic 3D micropatterned coculture, ES and HepG2 cells were stained with CellTracker Green and CellTracker Red (Invitrogen), respectively. For staining, the cells were treated with CellTracker working solution for 30 min under adhesion culture conditions. After being washed with DMEM, the stained cells were incubated under growth conditions appropriate for the particular cell type.

A calcein-AM/ethidium homodimer Live/Dead assay (Invitrogen) was used according to the manufacturer's instructions to assess cell viability within the hydrogels. Images of the encapsulated cells were taken with a Nikon TE 2000U camera and spot advanced software.

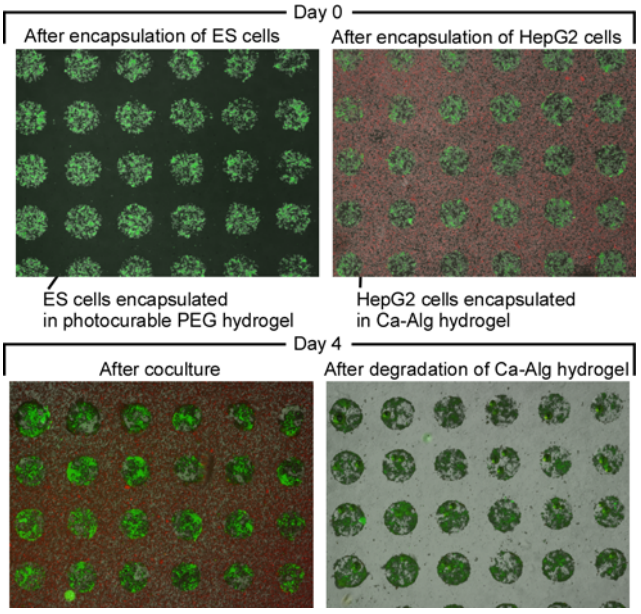

Figure 2: Dynamic 3D micropatterned coculture in photocurable-PEG and Ca-Alg hydrogels. ES and HepG2 cells were labeled with CellTracker green and CellTracker red, respectively.

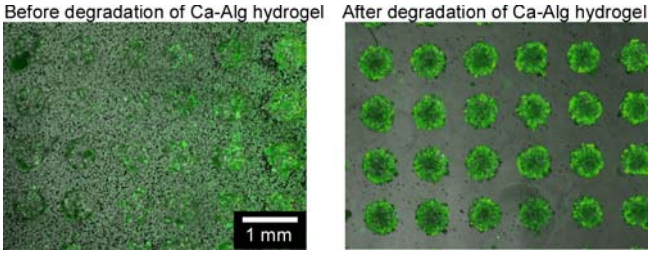

Figure 3: Live/dead assay through dynamic 3D micropatterned coculture.

Evaluation of beating activity

In the study of cardiac differentiation, PEG hydrogels containing beating colonies were counted on days 10, 12, 14, 16, 18, and 20 of culture. All 96 hydrogels in the entire area of the glass slides were examined for beating colonies, and the percentage of hydrogels containing beating colonies was calculated. Data were compared using ANOVA followed by Bonferroni's post-hoc test using GraphPad Prism 5.04 software.

RESULTS AND DISCUSSION

Dynamic 3D micropatterned coculture of ES cells with HepG2 cells was demonstrated by staining the cells with CellTracker reagent (Fig. 2). ES cells were encapsulated in the micropatterned PEG hydrogels and HepG2 cells were encapsulated in the Ca-Alg hydrogel arranged around the cell-laden PEG hydrogels (Fig. 2, Day 0). After cultivating these cells in 3D coculture condition for 4 days, the Ca-Alg hydrogel was degraded by exposure to sodium citrate solution, leaving the microarray of ES cells in PEG hydrogels. The 3D coculture environment around the ES cells could then be dynamically controlled (Fig. 2, Day 4). Dynamic 3D micropatterned coculture of ES cells with HepG2 cells was successfully demonstrated without any cross-contamination of the two different cell types. Most of the HepG2 cells were removed from around the PEG hydrogels after the Ca-Alg was degraded. ES cells could thus be cultivated without HepG2 cells for further differentiation experiments.

The encapsulated ES and HepG2 cells had good viability after encapsulation in the PEG and Ca-Alg hydrogels, respectively (Fig. 3, Day 0). The ES cells also had good viability after coculture for 4 days and degradation of the Ca-Alg hydrogel (Fig. 3, Day 4). The ES cells proliferated and migrated in the PEG hydrogels, and formed cell aggregates during coculture for 4 days.

We investigated the effect of dynamic coculture on the differentiation of ES cells along the cardiac lineage compared with continuous monoculture, that is, cultivation without HepG2 cells, and with continuous coculture (Fig. 4). In the 1st step culture, ES cells in coculture had better growth and were more likely to form cell aggregates compared with ES cells in monoculture. Also, we observed higher expression of FGF5, a gene marker for early-stage differentiation, and lower expression of Oct4, a gene marker for the undifferentiated state, compared with the monoculture (data not shown). These results indicate that soluble factors secreted by HepG2 are effective for

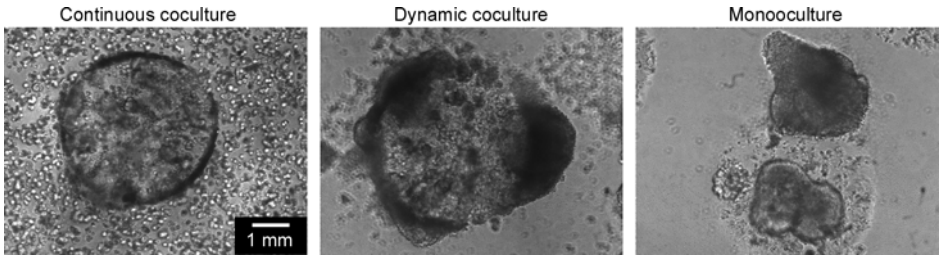

Figure 4: Microscope image of ES cells during the 2nd step culture (Day 16).

early-stage differentiation and maintenance, as has been reported previously [11, 12].

Under dynamic coculture conditions, the HepG2 cells in the Ca-Alg hydrogel were removed by treatment with citric acid after the 1st step culture. Then, the ES cells in the PEG hydrogels were cultivated in cardiac differentiation media. During the 2nd step culture, we observed better growth of ES cells in the dynamic coculture than in the continuous coculture (Fig. 4). Furthermore, we observed better differentiation along the cardiac lineage in the dynamic coculture compared with the continuous coculture and monoculture, as indicated by the higher percentage of beating colonies. This better differentiation was probably due to the induction of early-stage differentiation by the soluble factors secreted by the HepG2 cells in 1st step coculture and by the lack of nutrients and accumulation of metabolic waste in the continuous coculture due to the high cell density after the growth of the encapsulated cells.

CONCLUSION

We proposed the dynamic 3D micropatterned coculture of ES cells with HepG2 cells in photocurable PEG and chemically degradable Ca-Alg hydrogels. We demonstrated temporal control of the 3D microenvironment by degrading the Ca-Alg hydrogel. We observed higher cardiac differentiation of ES cells in the dynamic 3D micropatterned coculture compared with continuous coculture and monoculture. This method offers a convenient approach to engineering complex cell-cell interactions in a 3D tissue construct in a spatially and temporally regulated manner.

REFERENCES

[1] P. Zorlutuna, N. Annabi, G. Camci-Unal, M. Nikkhah, J. M. Cha, J. W. Nichol, A. Manbachi, H. Bae, S. Chen and A. Khademhosseini, *Microfabricated biomaterials for engineering 3D tissues*, Adv. Mater., 24, pp. 1782 (2012).

[2] H. Qi, Y. Du, L. Wang, H. Kaji, H. Bae and A. Khademhosseini, *Patterned differentiation of individual embryoid bodies in spatially organized 3D hybrid microgels*, Adv. Mater., 22, pp. 5276 (2010).

[3] J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar and A. Khademhosseini, *Cell-laden microengineered gelatin methacrylate hydrogels*, Biomaterials, 31, pp. 5536 (2010).

[4] T.M. Hammoudi, H. Lu and J. S. Temenoff, *Long-term spatially defined coculture within three-dimensional photopatterned hydrogels*, Tissue Eng. C, 16, pp. 1621 (2010).

[5] S. Khetan and J. A. Burdick, *Patterning network structure to spatially control cellular remodeling and stem cell fate within 3-dimensional hydro-gels*, Biomaterials, 31, pp. 8228 (2010).

[6] Y. Du, E. Lo, S. Ali, and A. Khademhosseini, *Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs*, Proc. Natl. Acad. Sci., USA, 105, pp. 9522 (2008).

[7] Z.J. Gartner, and C.R. Bertozzi, *Programmed assembly of 3-dimensional microtissues with defined cellular connectivity*, Proc. Natl. Acad. Sci., USA, 106, pp. 4606 (2009).

[8] J.W. Nichol, and A. Khademhosseini, *Modular tissue engineering: engineering biological tissues from the bottom up*, Soft Matter 5, pp. 1312 (2009).

[9] Y.T. Matsunaga, Y. Morimoto, and S. Takeuchi, *Molding cell beads for rapid construction of macroscopic 3D tissue architecture*, Adv. Mater. 23, H90 (2011).

[10] A. M. Kloxin, A. M. Kasko, C. N. Salinas, and K. S. Anseth, *Photodegradable hydrogels for dynamic tuning of physical and chemical properties*, Science, 324, pp. 59 (2009).

[11] J. Rathjen, and P.D. Rathjen, *Lineage specific differentiation of mouse ES cells: Formation and differentiation of early primitive ectoderm-like (EPL) cells*, Diff. Embryo. Stem Cell. 365, pp. 3 (2003).

[12] J. Rathjen, J.M. Washington, M.D. Bettess, and P.D. Rathjen, *Identification of a biological activity that supports maintenance and proliferation of pluripotent cells from the primitive ectoderm of the mouse*, Biol. Reprod. 69, pp. 1863 (2003).

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