IN VITRO GENERATION OF PANCREATIC PSEUDO-ISLETS USING FREE-STANDING MESH PATTERNED CELLULAR HYDROGEL

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ABSTRACT

This paper reports a simple and direct method to fabricate mesoscopic hydrogel constructs for size controlled pseudo-islets with MIN6 cells, a pancreatic β-cell insulinoma, *via* lamination of free-standing mesh patterned cellular hydrogel. The MIN6-laden hydrogels with three different micro-patterns were simply fabricated, free-standing cultured, and functionally analyzed to examine the effect of the microscale hydrogel patterns on pancreatic cellular growth. The presented system can be applied to an *in vitro* model for investigating cell and tissue morphogenesis in three dimensions (3D) and biofabrication of tissue constructs with microscale control of 3D cellular geometry, which could have great potential for the basic platform of functional pancreatic cellular assay.

KEYWORDS

Pancreatic pseudo-islets, Mesh patterned hydrogel, Cellular construct, MIN6 cells, Biofabrication

INTRODUCTION

Pancreatic β-cells are the principal source of hormone insulin, which maintains metabolic homeostasis. They proliferate and maintain *in vivo* as a spherical cellular group, called islets of Langerhans, that grows to approximately 100 μm. Therefore, both facultative *in vitro* β-cell proliferation and maintenance as a form of pseudo-islets is required for reducing a labor-intensive process and developing a cost-effective high-throughput assay as much as an animal model can provide a benefit. Not only was conventional hanging-drop technique introduced for β-cell reaggregation, but also the specialized hydrogel-based technology using optical setups has been recently developed to increase the biological function of pancreatic β-cell *in vitro* [1]. However, there are few simple methods for the size-controlled cellular constructs within the hydrogel platform without the cumbersome equipment. In this study, *via* fabrication of mesoscopic free-standing hydrogel platform [2], we present a simple method for formation of size-controlled pseudo-islets with MIN6 cells using micro-patterned hydrogel constructs, and analysis of their cellular function.

EXPERIMENTAL

As shown in Figure 1, the free-standing cellular hydrogel was fabricated by previously reported method [2]. To fabricate uniform mesh patterns, a poly(dimethylsiloxane) (PDMS) replica was fabricated *via* two-step lithography. To investigate the cellular effect of microscale patterns on hydrogel, three different conditions were examined; plain, block, and mesh patterns. Total loaded area (A) was defined and controlled by each pattern type ($A_{plain} = A_{block} = A_{mesh}$) to control the volume of hydrogel precursors including a certain number of cells.

Each PDMS substrate was modified by a plasma treatment to create a hydrophilic surface and a controlled volume (7~10 μL) of hydrogel precursors with MIN6 suspension (5×10^6 cells/mL) was introduced as a thin film (50~70 μm thick) by pipetting. 1% (w/v) sodium alginate in phosphate buffered saline (PBS) and 100 mM calcium chloride in distilled water were used. Then, the thin film of hydrogel precursor was cross-linked with a nebulized aerosol of gelling agent, *via* a nebulizer with an ultrasonic transducer. The fabricated cellular hydrogels were submerged into cell culture media and released from the substrate as free-standing units. Each cellular hydrogel was cultured with the same conventional culture media (Dulbecco's Modified Eagle Medium with 4.5 g/L glucose supplemented with 15% fetal bovine serum, 100 mg/L penicillin-streptomycin and 71.5 μM 2-mercaptoethanol) within a separated 24-well plate for 2 weeks. The secreted media were collected and frozen at −80 °C during a free-standing static culture period. Insulin secretion measurements were performed for day 3, 6, 9 and 12 using Rat/Mouse Insulin ELISA kit (Millipore, MO) according to the provided instruction. Insulin secretion level per single cell was calculated by a relative cell number which was daily measured by cell viability assay kit (Daillab, Korea).

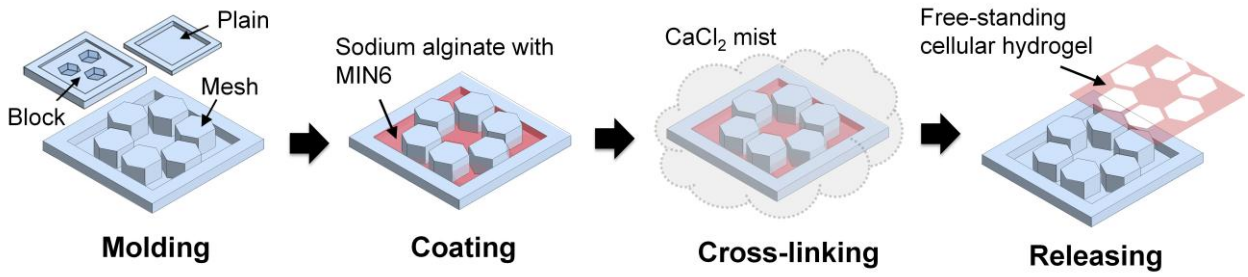

Figure 1. Schematic of free-standing patterned cellular hydrogel fabrication.

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RESULTS AND DISCUSSION

To verify the microscale patterns of hydrogel, methylene blue was stained with hydrogel only (Figure 2a) and hydrogel containing 6 μm-sized polystyrene beads (Figure 2b) with three different patterns; plain, block, and mesh patterns. The consistent block and mesh patterns were indicated through an intense blue color and microbead dispersion within hydrogel.

MIN6 cells were cultivated for 13 days within free-standing cellular hydrogels including three different patterns (Figure 2c–e). When the cells were loaded with hydrogel precursors, they were evenly distributed within the free-standing cellular hydrogel at day 1 (Figure 2c). In the course of time, however, cells were proliferated and took a controllable morphology as pseudo-islets *via* different molding patterns, especially mesh patterns (Figure 2e). To demonstrate their morphology, the cellular cytoplasm was visualized by green fluorescence (Figure 2f).

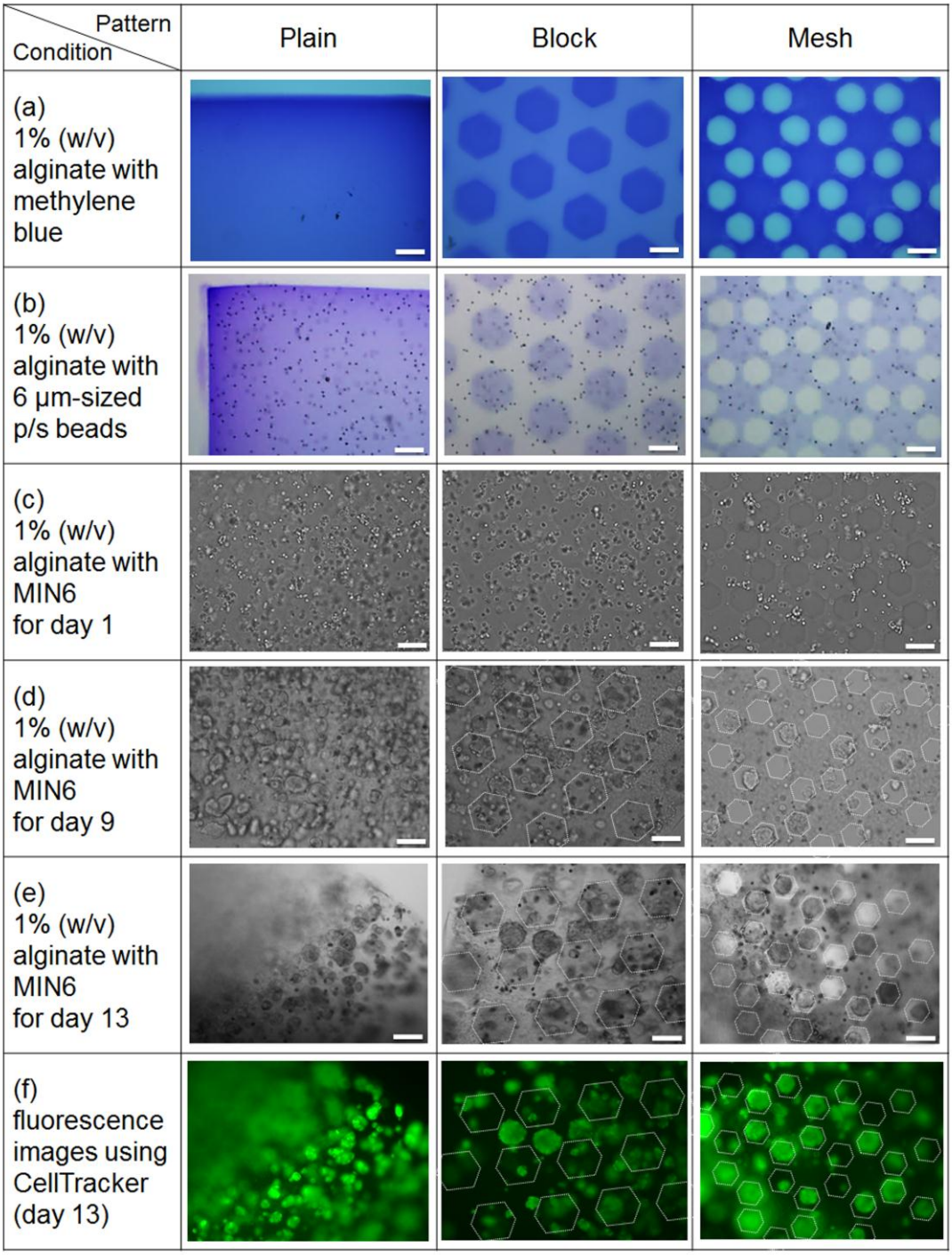

Figure 2. Bright-field and fluorescent images of fabricated free-standing hydrogel without any pattern (plain) or with block and mesh patterns. Methylene blue was stained with hydrogel only (a) and hydrogel containing 6 μm-sized polystyrene beads (b). Cellular hydrogels including MIN6 cells were cultured as a free-standing form at day 1(c), day 9(d) and day 13 (e). Fluorescent images using CellTracker in panel e (f). White mesh line indicates a pattern type. All scale bars are 100 μm.

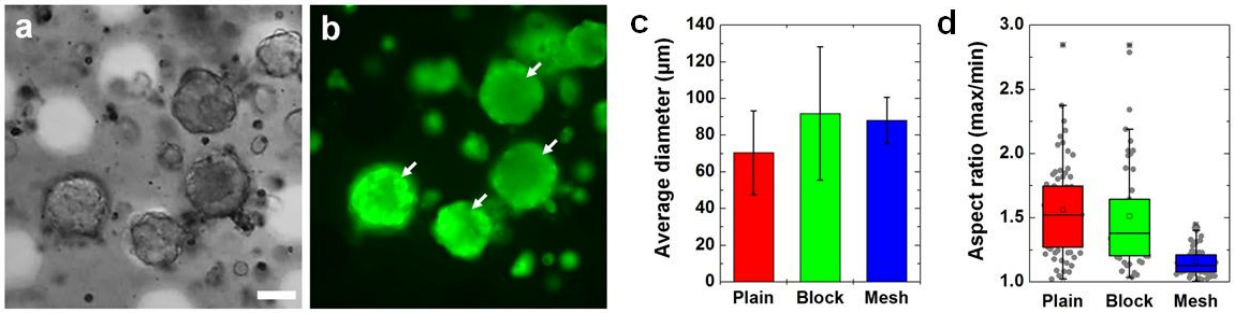

Figure 3. Morphological size analysis of pseudo-islets from the fabricated free-standing cultured cellular hydrogels. (a,b) Bright-field and fluorescent image for pseudo-islets from the free-standing mesh patterned cellular hydrogels (day 13). (c) Average diameter of pseudo-islets. (d) Aspect ratio between maximal and minimal width of pseudo-islets (n = 60). White arrow indicates a pseudo-islet. Scale bar = 50 μm.

To analyze morphology of multicellular lumps, both size and aspect ratio of multicellular units was measured *via* fluorescence images at culture day 13 (Figure 3a,b). According to their fluorescence images, most cells were proliferated to a self-assembly lump in the form of a pseudo-islet with plain and block patterns. On the other hand, they were not only proliferated to the lumps, but also taken their places at the empty spaces of the mesh patterned hydrogel. Although the average diameter of the pseudo-islets was comparable each pattern condition (Figure 3c), it was more homogeneous in the mesh patterns than that in other patterns. Moreover, the regular spherical units were observed more frequently in the mesh pattern than that in the block or plain patterns (Figure 3d).

To quantitatively evaluate the biological function of pseudo-islets within the micropatterned hydrogels, both cellular proliferation and insulin secretion was measured according to the culture days. Insulin secretion was approximately equivalent at the beginning of the culture period. However, insulin releasing level per a single cell in the mesh patterns was constantly increased with cultivation time compared to other types such as plain pattern (Figure 4).

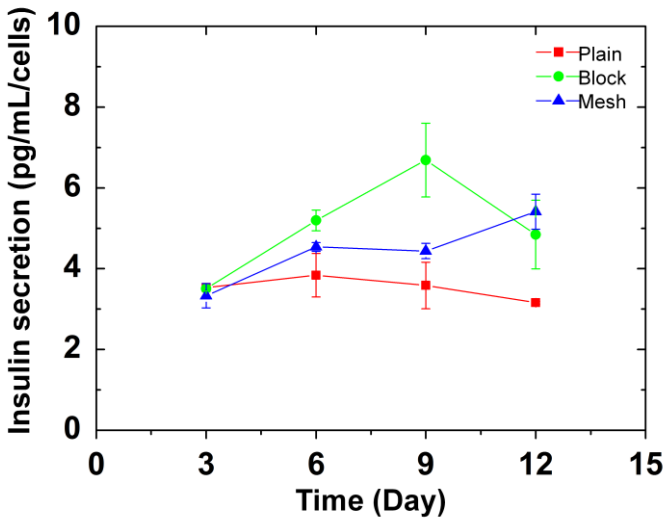

Figure 4. Insulin secretion level per unit cell of MIN6 cells from the free-standing cultured cellular hydrogels with plain, block and mesh patterns for 12 days.

CONCLUSION

In summary, we suggested a simple method to fabricate the mesoscopically micro-patterned free-standing cellular hydrogel for formation of size-controlled pseudo-islets, and quantitative analysis of their cellular function. The proposed system could be applied to an *in vitro* model for investigating 3D cellular geometry and have great potential for the basic platform of functional pancreatic cellular assay.

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