CELL ADHESION CONTROL INITIATE CELL SHEET FORMATION IN A MEDIUM SUSPENSION

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ABSTRACT

We have developed a novel technique for cell sheet formation and continuous cell culture without the need for frequent subculture that make use of open-mesh scaffold suspended in a culture medium. Epithelial cells seeded on the scaffold underwent elongation and proliferation normally, expanding dynamically to form a monolayer cell sheet over the meshed scaffold. Cytoskeletal labeling revealed extensively interconnected meshwork of the actin cytoskeleton, suggesting that the mechanical strength of the cell sheets was due to cell-cell adhesion. Thus the method provides a simple approach to cell sheet generation without the use of expensive smart polymers.

KEYWORDS: Tissue Engineering, Cell Sheet, Microfabrication, Open-Mesh Scaffold, Cell Adhesion Control

INTRODUCTION

Cell interaction with the surrounding extracellular cell matrix (ECM) determines various cell functions such as cell motility, proliferation and differentiation [1, 2]. Although microfabrication has enabled increased scalability with regard to cell adhesion control using micro-patterning and micro-contact printing techniques [3], methods of cell adhesion control on a continuous 2D surface suffer the inherent problem of non-specific cell adhesion arising from lateral diffusion of coated proteins and because some cells secret adhesion molecules over time.

As an alternative approach, this study presents a novel method for cell adhesion control where cells are seeded on a micro-fabricated scaffold with large open meshes and suspended in a culture medium such that cell adhesion is limited to the surface of 5 \(\mu\)m-wide micro-strands interspacing the open meshes. We demonstrate that epithelial cells can be seeded and grown on such a scaffold to form a monolayer cell sheets over the open meshes.

EXPERIMENTAL

The procedures for fabricating the meshed scaffold is shown schematically in Figure 1, and the device assembly and cell seeding procedures are shown schematically in Figure 2. To fabricate the scaffold, first a thin layer of gelatin was spin-coated on an electron beam-patterned photomask to form a sacrificial layer. Then SU-8-2 was spin-coated at 2000 rpm directly on top of the gelatin layer to give a film thickness of about 2 \(\mu\)m. After soft-baking for 1 min at 60°C and then for 3 min at 95°C, we performed UV exposure and development according to manufacturer’s instructions. Then the edges of the exposed areas were laminated with kapton tape (100 \(\mu\)m thick) for reinforcement, and the formed thin SU-8 sheet was detached from the photomask by immersion in hot water (90°C) to dissolve gelatin (Figure 1).

To ensure that cells were completely out of contact with the culture dish bottom while still permitting microscopic observation, the scaffold was completely raised off the dish bottom using silicon rubber spacers with a thickness of 500 \(\mu\)m (Figure 2). After incubation for 1 hour with 100 \(\mu\)g/mL fibronectin to promote cell attachment, mouse embryonic fibroblastic cells (MEF cells) and TIG120 cells were seeded on the suspended sheet and left undisturbed for 1 day to allow for cell attachment. Time-lapse microscopy was then performed to monitor cell behavior on the micro-strands.
RESULTS AND DISCUSSION

To monitor the dynamics of cells on the micro-strands, we live-stained MEF cells with CellLight-RFP (Molecular Probes) and performed time lapse microscopy. As shown in Figure 3A, MEF cells attached on the micro-strands of the meshed scaffold appeared elongated and crescent-shaped during the initial stages of attachment. Remarkably, these cells could detach, divide and then reattach back onto the narrow micro-strands and elongate again (Figure 3B-F). Intriguingly, after division, daughter cells would move in opposite directions to attach to the strands where the two ends of the mother cell were attached before division (Figure 3C-E). The mechanism for this is not clear but we postulate that the mother cells leaves behind some collagen threads as during retraction. Daughter cells might utilize this as a guide to move to attachment area.

![Figure 3: Time-lapse images of MEF cells undergoing division on an open-mesh scaffold suspended in a culture medium](image)

Remarkably, after culturing for 2 days, cells that were initially attached primarily at the intersections of the micro-strands began to spread and migrate, eventually forming a monolayer sheet over the open meshes of the scaffold, as shown in Figure 4. Depending on mesh shape and cell density, it took between 3-7 days to close a 200 μm-size mesh, with triangular meshes closing much faster than square meshes. To determine how cells could migrate and form a sheet over large-size open meshes (almost 5 times larger than a single cell), we monitored cell behavior in the vicinity of a closing hole such as that marked by a white dotted circle in Figure A (top). We observed that new cells from cell division

![Figure 4: MEF cells form cell sheet over an open-mesh scaffold](image)
would move toward the periphery of a closing hole, anchored by older cells that were already established on the micro-strands. Moreover, cells at the periphery of closing hole were observed to extend a meniscus-shaped membranous structure that acted as support to newly migrated cells. The circular shape of a closing hole is due to the contribution of surface tension in the closing a hole by providing support for cells to establish themselves at the hole periphery, leading to mesh closure. A combination of these events led to mesh closure, resulting in a uniform cell sheet over the open-mesh scaffold, as shown in Figure 4B for MEF cells and Figure 5A for TIG 120 cells.

Intriguingly, a continuous network of the actin cytoskeleton was found to exist over the entire cell sheet, suggesting that actin cytoskeleton is crucial to the mechanical strength of the sheet (Figure 5B,C). From Figure 5B,D, it can be observed that actin filament alignment is nearly uniform with each mesh, but varies from mesh to mesh. This is important in the case where cell sheet with heterogeneous cell alignment is required such for heart tissue regeneration. Overall, the formation process of an epithelial cell sheet over an open mesh mimic wound healing process, and is expected to aid in the understanding of this complex biological phenomenon [4].

CONCLUSION
This study has explored the generation of epithelial cell sheets using large open-mesh scaffold suspended in a culture medium. Results show that cells are capable of elongation and cell division on narrow (5 μm in width) micro-strands and can proliferate to form a uniform monolayer cell sheet over a mesh with a length-scale nearly 5 times larger than a single round cell. Actin network staining revealed that the cell sheet is supported by a continuous network of actin filaments, suggesting that the cell sheet is formed by cell-cell adhesion and interconnectivity. Thus, open-mesh scaffold method can be used not only for cell sheet generation, but also for long term culture of adherent cells.

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REFERENCES

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