The Shoji Technique for Cell Adhesion Control and Fabrication of Cell Sheets

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Thanks to rapid developments in microfabrications, it is now possible to engineer the cell culture substrates that closely mimic the in vivo environment, enabling detailed investigation of the influence of cell-substrate interaction on a wide variety of biological phenomena such as cell division, differentiation, morphogenesis, among others. Herein we present our newly developed cell culture platform, referred to as the "shoji technique", that employs suspended micro-meshes consisting of characteristically large apertures (> 100 μm in length dimension) and narrow mesh strands (3~5 μm in width) as substrates for cell culture. The meshes are microfabricated by photolithography and are set suspended in the culture medium (Fig. 1) for cell seeding and culture such that for cells on mesh, cell-substrate interaction is restricted to the narrow mesh strands. We demonstrate the capability of this culture method to modulate cell-substrate interaction by minimizing adhesion area, enabling easy fabrication of monolayer cell sheets by self-assembly mediated morphogenesis [1]. Cell sheets obtained by this method are mostly monolayer and, since they are formed in suspension, they can be harvested readily without any additional detachment procedure. Moreover, in the suspended position, cells are exposed to the culture medium from both sides, hence sufficient nutrient supply. Thus, cell sheets can be maintained for an extended period of time, simply by changing the cell culture medium periodically. Interestingly, mesh shape can be designed appropriately to control cell orientation, as demonstrated for the case of diamond-shaped meshes where cells align themselves in the direction of the longest axis (Fig. 2), suggesting that cell can sense and respond to the substrate geometry.

For application of the 2D cell sheets fabricated, on-going research is investigating the possibility of extending the "shoji technique" to 3D culture either by cell sheet stacking or by direct overlay culture of different cell types to mimic body tissues. Using this approach, we aim to fabricate tissue models that closely recapitulate the functionality of in vivo tissues. This is made possible by the fact that cell sheets generated by the shoji technique are able to permit cross-layer direct cell-cell interaction because the meshes have large apertures and thickness is only ~2 μm.

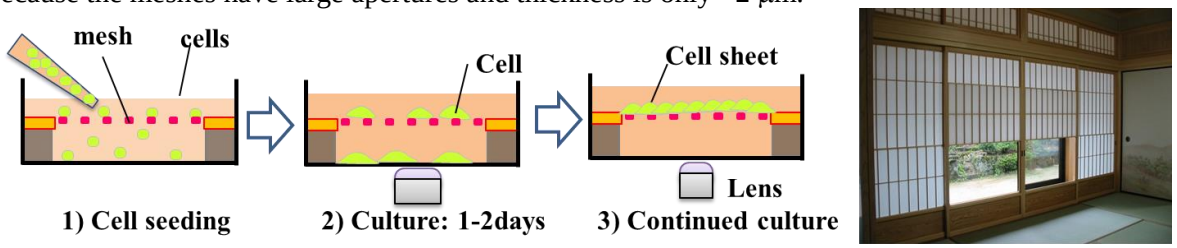

Fig. 1 Cell seeding on a suspended mesh leads to the formation of a cell sheet over the mesh by self-assembly organization. The process is analogous to fabricating "shoji" shown on the right.

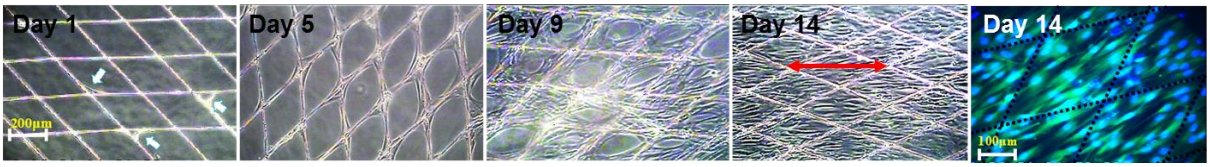

Fig. 2 Time course of cell sheet formation on a diamond mesh. Cell orientation was primarily in the longest direction, and cells were determined to be viable by calcein AM staining (far right panel): Scale bar is 200 μm for the left 3 images and 100 μm for the last 2 images.

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REFERENCES

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