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Micro-patterned cell-sheets fabricated with stamping-force-controlled micro-contact printing

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ABSTRACT

Cell-sheet-engineering based regenerative medicine is successfully applied to clinical studies, though cell sheets contain uniformly distributed cells. For the further application to complex tissues/organs, cell sheets with a multi-cellular pattern were highly demanded. Micro-contact printing is a quite useful technique for patterning proteins contained in extracellular matrix (ECM). Because ECM is a kind of cellular adherent molecules, ECM-patterned cell culture surface is capable of aligning cells on the pattern of ECM. However, a manual printing is difficult, because a stamp made from polydimethylsiloxane (PDMS) is easily deformed, and a printed pattern is also crushed. This study focused on the deformability of PDMS stamp and discussed an appropriate stamping force in micro-contact printing. Considering in availability in a medical or biological laboratory, a method for assessing the stamp deformability was developed by using stiffness measurement with a general microscope. An automated stamping system composed of a load cell and an automated actuator was prepared and allowed to improve the quality of stamped pattern by controlling an appropriate stamping force of 0.1 N. Using the system and the control of appropriate stamping force, the pattern of 8-mm-diameter 80-μm-stripe fibronectin was fabricated on the surface of temperature-responsive cell culture dish. After cell-seeding and cell culture, a co-culture system with the micro-pattern of both fibroblasts and endothelial cells was completed. Furthermore, by reducing temperature to 20 °C, the co-cultured cell sheet with the micro-pattern was successfully harvested. As a result, the method would not only provide a high-quality ECM pattern but also a breakthrough technique to fabricate multi-cellular-patterned cell sheets for the next generation of regenerative medicine and tissue engineering.

1. Introduction

For a decade, cell sheet transplantation is becoming one of key methods in regenerative medicine [1]. A cell sheet is a thin membrane composed of cultured cells and can be harvested from a temperature-responsive cell-culture dish by simply lowering temperature [2]. Since a cell sheet format is suitable for transplanting plenty of cells onto the surface of tissues/organs like a patch, cell sheets are widely used for repairing the damaged tissues/organs such as the skin [3], cornea [4], myocardium [5], esophagus [6], lung [7] periodontal tissue [8], cartilage [9], and middle ear mucosa [10]. These cell sheets include only a single type of cells, and for example, epithelial cells are used for repairing the skin, cornea, and esophagus, and muscle cells are used for the myocardium. On the other hand, although a cell-sheet imitating complex tissue such as liver [11] etc. have been attempted to be fabricated in several laboratories [12–15], they have been never used for actual clinic. Upon the demand of repairing the damaged complex tissues with cell sheets, a patterned multi-type-cell sheet is an essential material with a potential for transplants.

One of useful techniques for fabricating complex cell sheets is a protein patterning by micro-contact printing [16] and consequent cell spontaneous organization by the adhesion interaction between cells and proteins in extracellular matrix (ECM) [17]. In micro-contact printing, a protein on a stamp with a target pattern made from polydimethylsiloxane (PDMS) is transferred to the surface of cell culture dish. Generally, micro-contact printing is still manually operated [18]. However, this technique requires highly skillful

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technicians who can adjust the stamping force adequately, because PDMS stamp is easily deformed by the stamping force [19]. When the deformation of stamp is too large to exceed the height of stamp pattern, of cause, the bottom of stamp attaches on the dish surface, resulting in an unsuccessful printing with over-size patterns. Therefore, ECM patterning by an automated system is highly demanded. When the automated system is used, an index for determining an appropriate stamping force should be required, and considered the relationship between stamping force and the deformation of PDMS stamp, namely the stiffness of PDMS stamp.

The effect of automated-device use is expected to improve the accuracies of stamping-force measurement and positioning PDMS stamp to an object surface with a level far higher than that of manual operation. Therefore, for improving the quality of printed ECM pattern, the combination of load cell, a kind of force sensor, and an automated stage with a positioning accuracy of micrometer order has been proposed to be a useful system in the authors' previous study [20]. The previous study also proposes methods for calibrating the stiffness of PDMS stamp and estimating an appropriate stamping force, and performs the preliminary experiment of micro-contact printing in a stamping force measurement. However, the fabrication of patterned co-cultured cell sheet never succeeds because of inadequate experimental conditions. Furthermore, there is no discussion about the applicable range of the calibration method of stamp stiffness. Therefore, this study improved (1) an automated system for micro-contact printing and (2) the calibration method with considering in the stiffness of PDMS stamp for the automation of micro-contact printing for increasing its applicability. First, a stiffness measurement method capable to evaluate the stiffness of stamp without any contact was introduced with the higher precision of measurement than that in the previous study. Then, the automated system was basically improved with (1) a fixation device keeping both PDMS stamp and cell culture dish in parallel and (2) the replacement of force sensor into a precise load cell. An equation for the index of stamping force was derived with the stiffness of stamp, and its efficacy was verified by using the automated system controlling the stamping force with various levels with a minimum resolution of 0.1 N. Finally, this study showed the fabrication of co-culture system that had a pattern with both endothelial cells and fibroblasts based on the stamping-force-controlled micro-contact printing of ECM (Fig. 1).

2. Materials and methods

2.1. Fabrication of PDMS stamp

PDMS stamps were fabricated by a modified method as the previous study [19]. Silicon wafers (p-type, approxi. 75 mm in diameter, 380 μm in thickness) (SEMITEC, Chiba, Japan) were treated with vacuum oxygen plasma for 3 min at an intensity of radio frequency of 400 W and oxygen pressure of 13 kPa in a chamber by using a

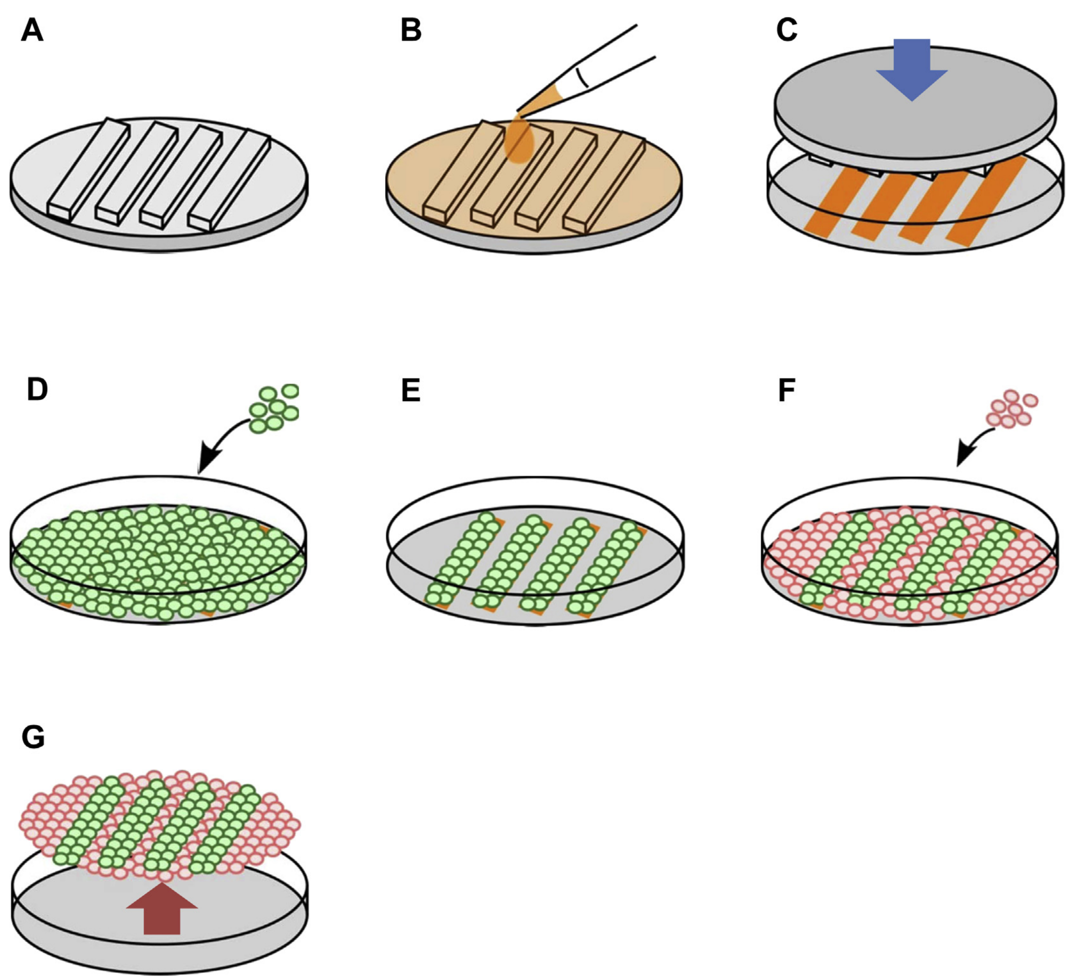

Fig. 1. Procedure for the fabrication of microstructured cell sheet by microcontact printing with stamping-force control. (A) Fabrication and inspection of polydimethylsiloxane (PDMS) stamp. (B) Application of extracellular matrix onto the surface of PDMS stamp. (C) Microcontact printing with appropriate stamping force. (D) Seeding the 1st cells. (E) Brief incubation and medium change. (F) Seeding 2nd cells. (G) After brief incubation, medium change, and cell culture, the harvest of cell sheet by reducing the temperature of temperature-responsive cell culture dish.