Cell Micropatterning Using Magnetite Nanoparticles and Magnetic Force

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Introduction

Tissue engineering is a promising technological approach to addressing shortages of organs available for transplantation. Tissue engineering aims to create functional organs by using biomaterials and cells. Artificial tissues can be implanted in patients to recover tissue functions that were lost due to severe injury, disease. However, it is difficult to construct functional organs because tissue-engineered architectures are not entirely similar to the *in vivo* organs, in which the cells are allocated precisely. Therefore, tissue engineering requires novel technology for allocating cells precisely to fabricating functional tissue architectures. One approach toward creating such a complex organization is the micropatterning of cells. Several cell patterning methods such as microcontact printing or lthography have been developed. However, these methods require cell patterning on specialized surfaces, and fabrication of surfaces is time-consuming. In the present study, we demonstrated cell micropatterning using magnetite nanoparticles and magnetic force, which is a novel technique able to easily allocate cells on arbitrary non-absorbing surface, including biological gels. We previously developed magnetite cationic liposomes (MCLs), which are cationic liposomes containing 10-nm magnetite nanoparticles [1]. MCLs can be taken up easily by target cells. The magnetically labeled cells can be manipulated by means of magnetic force, and we have developed an original tissue engineering methodology using the cells that were magnetically labeled by MCLs and magnetic force and have designated “magnetic force-based tissue engineering” (Mag-TE). With this technique, we fabricated mono-layered cell sheets and multi layered 3D construct without using any artificial polymer scaffolds [2, 3] and tubular constructs [4]. In the present study, we developed a novel methodology of cell patterning by using the Mag-TE technique, which can fabricate several cell patterns easily and rapidly with a high resolution.

Materials and Methods

Mouse NIH/3T3 fibroblasts (FBs) and Human umbilical vein endothelial cells (HUVECs) were cultured at 37°C under a humidified atmosphere of 5% CO_2 and 95% air in Dulbecco’s Modified Eagle Medium (DMEM) or commercially available growth media (HuMedia-EG2), respectively. Magnetite (Fe_3O_4; average particle size, 10 nm; Toda Kogyo, Hiroshima, Japan) was used as the core of the MCLs. The MCLs were prepared using colloidal magnetite and a lipid mixture as described previously [1].

FBs or HUVECs were cultured until subconfluent, and the medium was replaced with fresh medium containing MCLs (net magnetite concentration, 100 pg/cell). After

culturing with MCLs (culture time: FBs, 4 h and HUVECs, 24 h), magnetically labeled cells were harvested, and allocated by Mag-TE technique.

A steel plate with a thickness of 200 μm, a length of 30 mm, and a height of 2 mm was used for cell micropatterning (Fig. 1A). The procedure of cell micropatterning is illustrated in Fig. 1B. The steel plates were placed on a cylindrical neodymium magnet. The magnetically labeled cells were seeded into a culture dish, and the dish was then placed on the steel plates with a magnet.

To investigate the effects of the number of cells seeded on the width of the patterned lines, the seeding concentration of FBs was changed (5.0×10^3 ~ 5.0×10^5 cells/dish); the dish was then placed on steel plates with the magnet and was shaken slightly. To fabricate several cell patterns, the magnetically labeled FBs were seeded either into a dish on “curved” steel plates with a magnet or on “parallel placed” steel plates (200 μm apart) with the magnet. To fabricate the “crossing” pattern, magnetically labeled FBs were seeded into a dish on straight steel plates with the magnet and cultured for 30 min to allow the cells to attach onto the surfaces where the steel plates were positioned. The dish was then rotated and the magnetically labeled FBs were subsequently seeded into the dish, followed by medium change, and then cultured for 30 min.

Magnetically labeled HUVECs were seeded onto the gelated Matrigel on straight steel plates either set with the magnet or without the magnet (control). The cells were cultured for 1 d to induce angiogenesis. We used a confocal laser microscope to observe cross-sections of patterned HUVECs labeled by Cell Tracker (Molecular Probes, Eugene, OR).

Magnetically labeled HUVECs were seeded into culture dishes that did not facilitate cell attachment, and the steel plate with the magnet was placed under the culture dish. The dishes were used to harvest patterned cells without enzymatic treatment. After culturing of 1 d on the culture dish, the magnet was detached from the dish, and the dish was shaken slightly, and the patterned cells were manipulated with tweezers.

Results and Discussion

A steel plate was used for line patterning of target cells. The steel plates were placed on a cylindrical neodymium magnet. Magnetically labeled FBs were seeded into cell culture dishes, and the dish then placed on the steel plates with a magnet. After 30 min of culture, the cells allocated on the surface above the steel plates, and cells formed a straight line pattern (Fig. 2A, 2B). Furthermore, the line widths of the patterned cells could be adjusted by changing the cell concentration (Fig. 2C). Next, we investigated whether several patterns could be formed. Since a steel plate was sufficiently flexible to be bent, FBs in a curved line pattern were also observed (Fig. 3A). Further, since the steel plates were easy to handle, two steel plates were placed at a distance of 200 μm (Fig. 3B). More complex patterns, such as crossing patterns, were also constructed by sequential patterning (Fig. 3C). A remarkable point is that magnetic force-based cell patterning was not limited by the property of culture surfaces, and any culture surface may be used,

including biological gels and non-absorbing surfaces. Matrigel has often been used for angiogenesis assays, but fabrication of cell patterning on biological gels is difficult. In this study, HUVECs were patterned on Matrigel. HUVECs as well as FBs were allocated in a line where a steel plate was positioned (Fig. 4A). Moreover, after 1 d of culture on Matrigel, the cells elongated and formed connected capillary-like structures (Fig. 4B). Confocal microscopy revealed that lumens were formed into the capillaries (Fig. 4C), suggesting that they underwent early angiogenesis. When HUVECs that were magnetically labeled with MCLs were seeded at the same cell concentration without a magnet, the cells did not form capillary networks due to the lack of cell density (Fig. 4D). These results suggest that magnetic force-based patterning of HUVECs may be a feasible approach for fabricating functional vascularized organs.

As mentioned above, cell patterning using magnetic force may not limit the property of culture surfaces. Therefore, we conducted patterning of HUVECs on a non-absorbing surface. When magnetically labeled HUVECs were seeded and cultured for 1 d on a steel plate with the magnet, the cells aggregated and formed cord-like structures of HUVECs (Fig. 5A, 5B). When the magnet was removed, the cord-like structures detached from the surfaces and immediately floated to the surface of the culture medium (Fig. 5C). The cord-like structures of HUVECs had sufficient strength to be manipulated with tweezers (Fig. 5D), suggesting that the 3D cell-cell interactions were formed among the cells by 1-day cell cultivation in a 3D manner by using magnetic force. These results suggest that this novel methodology is a feasible approach for fabricating tissue-engineered tissue-engineered vascular structures.

In conclusion, we developed a novel methodology to pattern cells easily with a high resolution by using magnetite nanoparticles and magnetic force. We believe that this method could have potential applications in tissue engineering and provide a tool for the *in vitro* study of cell-cell interactions such as tissue vascularization.

Reference

[1] Shinkai M, Yanase M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T. Intracellular hyperthermia for cancer using magnetite cationic liposomes: in vitro study. Jpn J Cancer Res 1996;87(11):1179-83.

[2] Ito A, Ino K, Hayashida M, Kobayashi T, Matsunuma H, Kagami H, Ueda M, Honda H. Novel methodology for fabrication of tissue-engineered tubular constructs using magnetite nanoparticles and magnetic force. Tissue Eng 2005;11(9-10):1553-61.

[3] Ito A, Ino K, Kobayashi T, Honda H. The effect of RGD peptide-conjugated magnetite cationic liposomes on cell growth and cell sheet harvesting. Biomaterials 2005;26(31):6185-93.

[4] Ino K, Ito A, Kumazawa H, Kagami H, Ueda M, Honda H. Incorporation of capillary-like structures into dermal cell sheets constructed by magnetic force-based tissue engineering. J. Chem. Eng. Japan *subimitting*

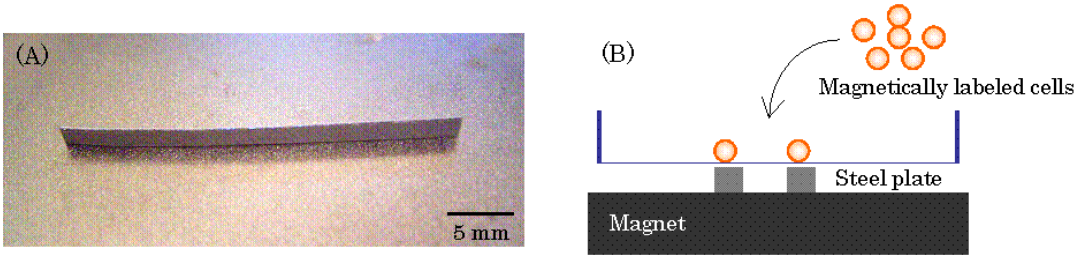

FIG. 1. Procedure for cell patterning by using magnetic force and magnetite nanoparticles. (A) A steel plate. (B) Illustration of cell micropatterning on the steel plates with a magnet. Magnetically labeled cells were seeded into a culture dish, and the dish was placed on the steel plates with the magnet for cell patterning, and then the dish was slightly shaken. The cells were attracted to the culture surfaces where a steel plate was positioned.

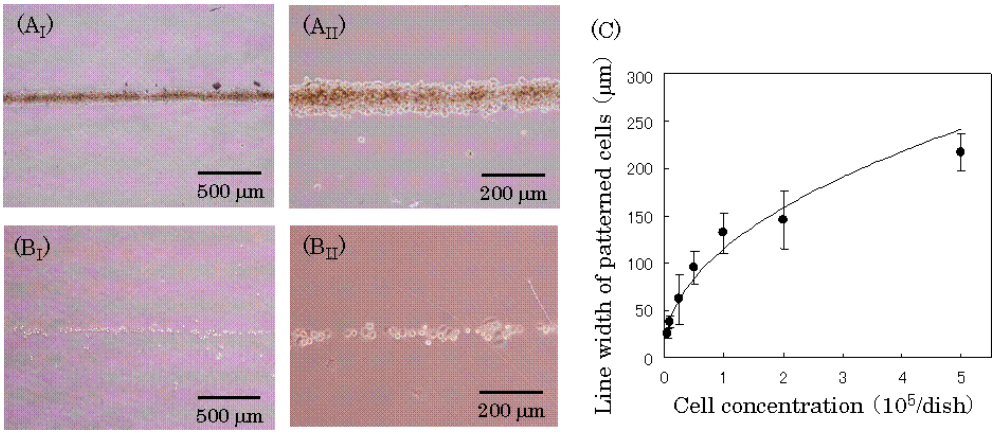

FIG. 2. Cell patterning using steel plates and magnets. The FBs were allocated on the surface where the steel plates were positioned (cell concentration; AI, 1.0 × 10^5 cells/dish; BI, 1.0 × 10^4 cells/dish). Enlarged photographs of parts AI and BI are shown in parts AII and BII, respectively. (C) The Line width of the patterned FBs in each cell concentration. Data points represent the means ± SD of six independent experiments.

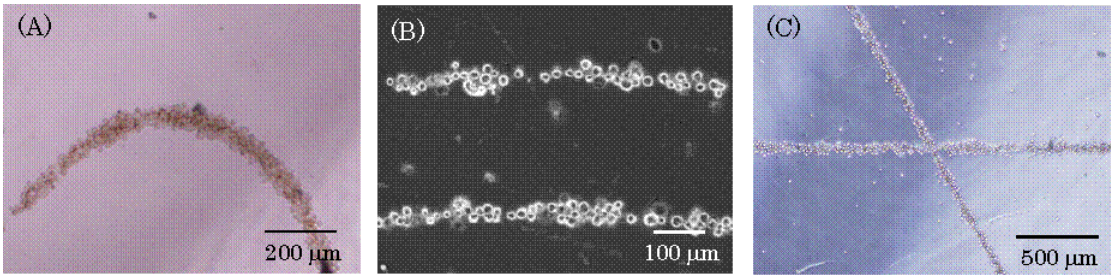

FIG. 3. Phase-microscope images of complex cell patterns. FB curve patterns (A) or FB parallel patterns (B) were fabricated. (C) FB line patterns were obtained by overlaying cells and the crossing patterns were fabricated.

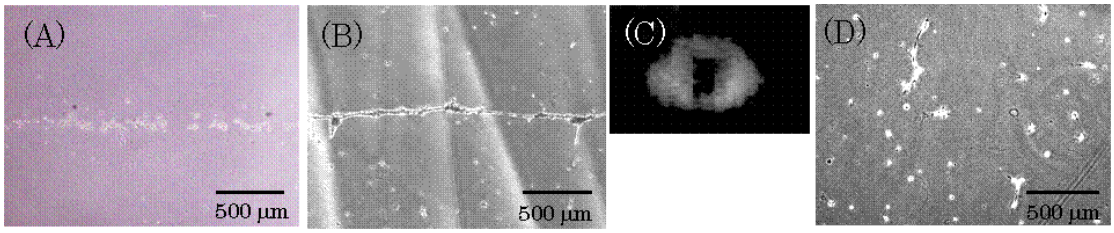

FIG. 4. Patterning of HUVECs on Matrigel. Magnetically labeled HUVECs were allocated on the surface where the steel plates were positioned. Phase-microscope images of patterned HUVECs at 30 min (A) or 1 d (B) after seeding the cells on a magnet. (C) Cross-section of patterned HUVECs was analyzed using a confocal laser microscope. (D) HUVECs were seeded on Matrigel without a magnet (control), and after 1 d of culture the cells were observed.

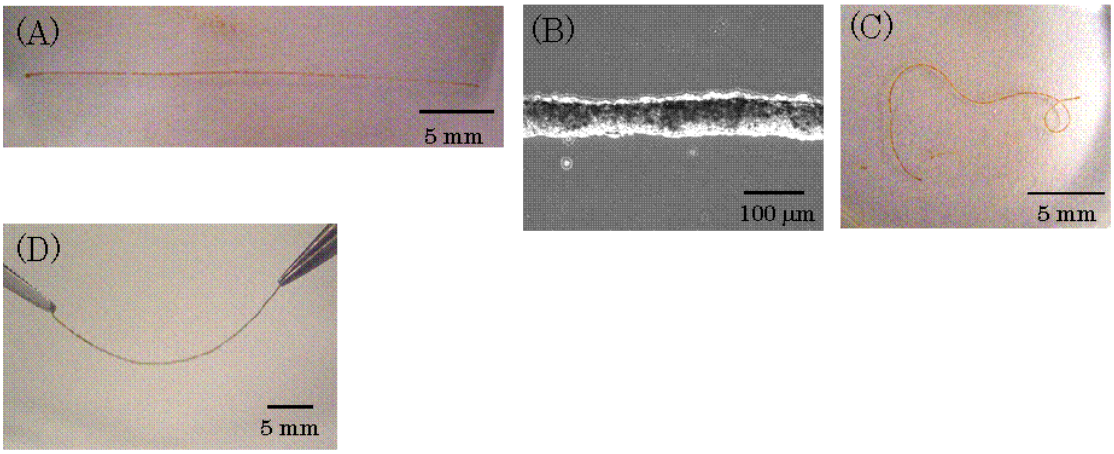

FIG. 5. Construction and harvesting of cord-like cell structures. Magnetically labeled HUVECs were seeded on non-absorbing surfaces positioned on straight steel plates with a magnet, and after a 1-d culture, cord-like structures were fabricated (A). (B) The cord-like structures were observed by phase microscope. When the magnet was removed, the cord-like structures were detached from the bottom of the cell culture surface and floated towards the surface (C). The cord-like structures of cells were harvested using tweezers (D).