Simple Magnetic Cell Patterning Using Streptavidin Paramagnetic Particles

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A simple methodology for cell patterning has been developed that can potentially be used to position different types of mammalian cells with high precision. In this method, cell membrane proteins were first biotinylated and then bound to streptavidin paramagnetic particles. The magnetically labeled cells were then seeded onto culture dishes and patterned using low magnetic fields. Highly defined cell patterns were achieved using HeLa, TE671 cells and human monocytes. HeLa and TE671 cells were also sequentially patterned and successfully co-cultured on the same plate using this technique. Cell viability studies proved that this magnetic labeling method was not toxic to cells. Transmission electron microscopy showed that the magnetically labeled HeLa and TE671 cells internalized some of the paramagnetic particles after two days of culture, while the labeled human monocytes did the same after only one hour. Uptake of these particles did not affect the cell patterning and cell viability. This magnetic labeling process is fast, as it involves affinity-based attachment of paramagnetic particles and does not rely on cellular uptake of magnetic materials. It may be adaptable and scalable for various applications. Exp Biol Med 234:332–341, 2009

Key words: cell patterning; magnetic cell labeling; paramagnetic particles; biotin; streptavidin; co-culture

Introduction

The ability to locate cells with positional precision enables fundamental studies to be done on cell-cell interactions and the impact of extra-cellular environment on cell proliferation (1). Moreover, the creation of well-organized cellular arrangements has numerous applications such as cell assembly in tissue engineering (2), fabrication of cell arrays for bio-sensing (3) and study of the cellular mechano-transduction process (4). Beyond merely patterning cells, the capability to direct cells can also benefit cell based therapies such as the use of stem cells to treat diseased organs or tissues (5).

There have been some advances in the development of technologies for precise positioning of cells. For example, soft lithography can be used to treat surfaces to control cell deposition (6) and it usually requires complicated procedures or specialized equipment. Cell patterning has also been achieved using an adapted inkjet printer (7), but the harsh treatment of this approach may cause physical damage to cells.

Magnetic particles have found increasing use in biomedical applications, such as magnetic resonance imaging (MRI), targeted drug delivery and magnetic separations (8). In particular, paramagnetic particles are magnetized in the presence of magnetic fields, but lose their magnetization when the fields are removed. More recently, magnetic cell patterning has been developed to direct magnetically labeled cells using magnetic fields. Ino *et al.* (9) labeled cells with magnetite cationic liposomes (MCLs) and Ito *et al.* (10) coupled Arg-Gly-Asp (RGD) to MCLs to promote cell attachment, while Sasaki *et al.* (11) used chitosan-coated magnetic nanoparticles. In the first two cases, the magnetic entities were taken up by the cells, while in the third, they were localized on the cell membranes. A non-specific labeling method used anionic magnetic nanoparticles to promote electrostatic adsorption to cell membranes before being taken up within endosomes (12). Tanase *et al.* (13) labeled cells by using ferromagnetic nanowires to bind non-specifically to cellular surfaces and

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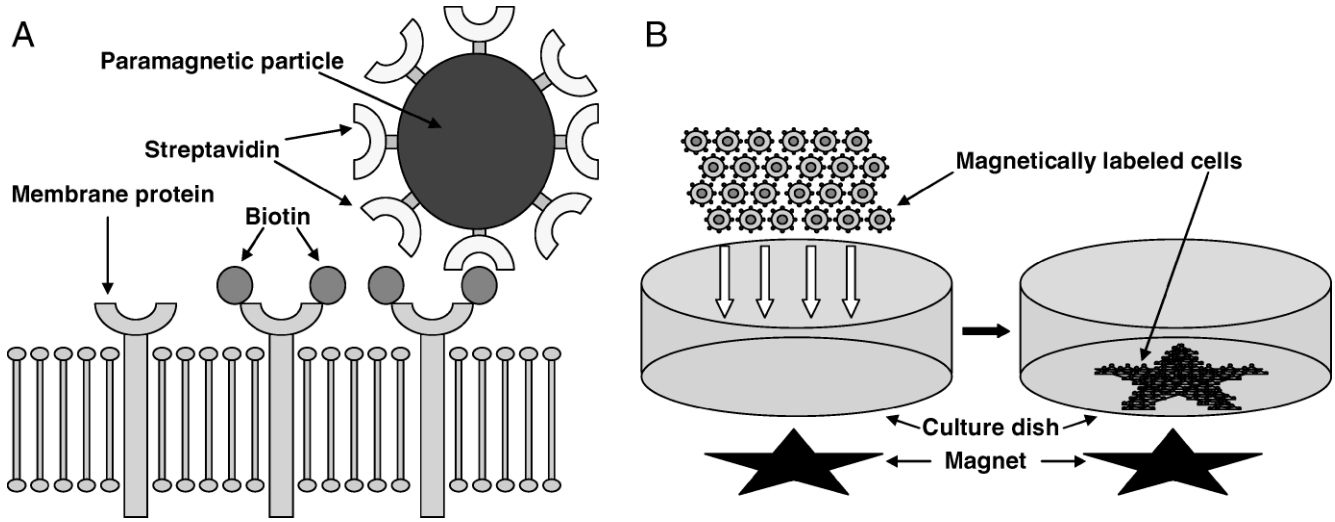

Figure 1. Schematics of the procedure for magnetic cell labeling and patterning. **A:** Magnetic cell labeling. Cell membrane proteins were first biotinylated and subsequently labeled with streptavidin paramagnetic particles. **B:** Magnetic cell patterning. A star-shaped magnet was attached under the culture dish. Magnetically labeled cells were added and patterned onto the plate by the magnetic field.

manipulate the labeled cells using magnetic fields to create microarrays of cells. In a more specific application, Perea *et al*. (14) utilized immunomagnetic labeling to label cells with magnetic microbeads and directed them with radial magnetic fields for seeding onto tubular scaffolds, in order to grow multi-layered cellular constructs for potential vascular tissue engineering applications.

Magnetic cell patterning is thus a promising technology, but it can be further improved by increasing the ease and rate of magnetic cell labeling and by adapting it for any cell type. Many of the current labeling methods involve cellular uptake of magnetic materials; however, the rate of uptake usually varies across cell types and the quantity of uptake cannot be easily controlled. Alternatively, immunomagnetic labeling is dependent on the number of specific receptors on cells and is limited to certain cell types. A labeling method that has broad applicability across cell types and is uptake-independent would simplify the process of magnetic cell patterning and widely extend its use (15).

The non-covalent binding between biotin and streptavidin is one of the strongest biological interactions and it occurs rapidly with considerable stability in a broad range of pH and temperature (16). It has been utilized in numerous applications such as purification of retroviral vectors (17, 18). The specificity of this interaction has made these applications possible. Here, a simple methodology is described in which cell surfaces are biotinylated and labeled with streptavidin paramagnetic particles. This process is expedient and the magnetically labeled cells are then patterned using magnetic fields with high precision.

Materials and Methods

Materials. Biotinamidohexanoic acid N-hydroxysuccinimide ester (BiotinSE, Sigma B2643), 366 mM in DMSO, was stored at –20°C. Ethylenediaminetetraacetic acid solution, 0.02% in Dulbecco's Phosphate Buffered Saline (EDTA, Sigma E8008) was stored at 4°C. Streptavidin MagneSpheres paramagnetic particles (Promega Z5482), 1 mg/ml (5×10^8 particles/ml) of 0.5–1.5 μm diameter particles were stored at 4°C. Bisiflex II magnetic sheeting with 0.75 mm thickness and with a pull force of 44 g/cm^2 (Bisbell Magnetic Products Limited, Burton-On-Trent, UK). Adhesive backed magnetic rubber sheet with 1.5 mm thickness and with a pull force of 68 g/cm^2 (e-magnets UK, Sheffield, UK). Neodymium Iron Boron (Nd-Fe-B) rectangular block magnet with dimensions of 40 mm $\times$ 20 mm $\times$ 10 mm and with a pull force of 19.9 kg (e-magnets UK, Sheffield, UK).

Cell Lines. HeLa human cervix adenocarcinoma cells and TE671 adherent human rhabdomyosarcoma cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Sigma D5796) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin, at 37°C and in a 5% CO_2 humidified atmosphere.

Isolation and Culture of Human Monocytes (HMs). HMs were isolated from human buffy coat residues (National Blood Service, Brentwood, UK) as described by Müller *et al.* (19). Briefly, monocytes were enriched by Ficoll and Percoll density centrifugations, washed with Phosphate Buffered Saline (PBS), and subsequently used for the magnetic labeling protocol as described below. Cells were cultured in Macrophage Serum-Free Medium (Mø-SFM, Invitrogen), containing 100 U/ml penicillin and 100 μg/ml streptomycin. HMs are adherent cells and they do not proliferate in culture.

Biotinylation and Magnetic Labeling of HeLa and TE671 Cells. A schematic of the procedure is given in Figure 1A. HeLa or TE671 cells were seeded at 1×10^6 cells in 10 ml fresh medium in T-75 culture flask and cultured over 72 h. Thereafter, the medium was thoroughly

aspirated and replaced with 5 ml PBS. After the PBS wash, the PBS was removed and replaced with 10 ml of freshly diluted BiotinSE (500 μM in PBS). After incubation at room temperature for 30 mins, the excess BiotinSE was aspirated and replaced with 10 ml medium to quench the reaction. The medium was removed and replaced with 5 ml PBS. The PBS was then replaced with 5 ml EDTA solution and the biotinylated cells were incubated for 15 mins at 37°C for cell detachment. To dilute the EDTA solution, 5 ml of medium was added. A cell count was then performed and the cells were spun down using a centrifuge and resuspended in fresh medium.

To prepare 4×10^6 magnetically labeled HeLa or TE671 cells, 0.4 ml of streptavidin paramagnetic particles were added to a 1.5 ml Eppendorf tube and applied to a Dynal MPC-S magnetic particle concentrator, the supernatant was removed and the particles were resuspended in 1 ml sterile PBS + 0.1% Bovine Serum Albumin (BSA). The suspension was then re-applied to the particle concentrator and the supernatant was aspirated. The particles were washed three times using this procedure and then suspended in 1 ml of fresh medium. 4×10^6 biotinylated cells in 7 ml medium were added to the paramagnetic particles and vortexed briefly at room temperature to ensure uniform mixing of cells and particles. The target ratio of added paramagnetic particles to cells for the magnetic labeling was 50:1.

Biotinylation and Magnetic Labeling of HMs. 4.8×10^7 HMs were biotinylated in suspension using 10 ml of diluted BiotinSE (500 μM in PBS) for 30 mins at room temperature. Thereafter, to quench the reaction, 5 ml of FBS was added and the cells were centrifuged. The supernatant was removed and the cells were re-suspended in fresh Mø-SFM.

To prepare 3.2×10^7 magnetically labeled HMs, 0.96 ml of streptavidin paramagnetic particles were washed in the same manner as described above. The particles were then suspended in 1 ml of fresh Mø-SFM. 3.2×10^7 biotinylated HMs in 7 ml Mø-SFM were added to the paramagnetic particles and vortexed briefly at room temperature to ensure uniform mixing of HMs and particles. The magnetically labeled HMs were added to six 1.5 ml Eppendorf tubes and applied to a Dynal MPC-S magnetic particle concentrator, the supernatant was removed and the HMs were resuspended in 1 ml PBS for each Eppendorf tube. The suspensions were then re-applied to the particle concentrator and the supernatant was aspirated and then all the HMs fractions were resuspended in 8 ml fresh Mø-SFM. The target ratio of added paramagnetic particles to HMs for the magnetic labeling was 15:1.

Cell Viability Studies. HeLa and TE671 cells were magnetically labeled, as described above. Control cells were biotinylated, but not labeled with paramagnetic particles. Thereafter, the cells were seeded at a density of 0.01×10^6 cells/well in 48-well tissue culture plates in fresh medium. One plate was placed in the incubator on a piece of magnetic sheeting (Bisiflex II, 22 cm × 27 cm), whereas the control plate was placed without a magnetic sheeting. After 72 h, samples were taken for the trypan blue exclusion assay. The total number of cells and number of non-viable cells were counted using an improved Neubauer haemocytometer. The cell viability data were statistically analyzed with Analyse-iT™ software using one-way ANOVA followed by a LSD post-hoc test.

Magnetic Cell Patterning of HeLa and TE671 Cells. A schematic of the magnetic cell patterning procedure is shown in Figure 1B. 1×10^6 magnetically labeled HeLa or TE671 cells were added to a 92 mm culture dish with a plate thickness of 1.65 mm and one magnetic star shape (Bisiflex II) was attached to the bottom to pattern the cells. Three controls were also prepared. In the first control, 1×10^6 magnetically labeled cells were added to a 92 mm culture dish in the absence of a magnetic star shape. In the second control, 1×10^6 non-labeled biotinylated cells were added to a 92 mm culture dish in the presence of a magnetic star shape. In the third control, 1×10^6 non-biotinylated cells, briefly vortexed with streptavidin paramagnetic particles (target ratio of 50 particles/cell), were added to a 92 mm culture dish in the presence of a magnetic star shape. All experiments and controls were conducted in duplicates and all cultures were made up to 10 ml fresh medium. The cultures were then agitated for 30 mins at room temperature, after which they were returned to 37°C. Medium change was performed daily for all samples and the cells were cultured for 72 h.

For the magnetically controlled co-culture of HeLa and TE671 cells, 2×10^6 magnetically labeled TE671 cells in 10 ml medium were added to a 92 mm culture dish which was placed on top of the Nd-Fe-B rectangular block magnet. The culture dish and magnet were incubated at 37°C for 24 h. The rectangular magnet was then rotated by 90° and the medium was removed. 2×10^6 magnetically labeled HeLa cells in 10 ml fresh medium were added to the same dish and incubated at 37°C with the magnet in this new orientation for 24 h.

Magnetic Cell Patterning of HMs. 8×10^6 magnetically labeled HMs were added to a 35 mm well with a plate thickness of 1.65 mm in 6-well cluster plate and one magnetic star shape (adhesive backed magnetic rubber sheet) was attached to the bottom to pattern the cells. Similarly, two controls were prepared as in the patterning experiments for HeLa and TE671 cells; one with the magnetic star shape absent and one with the non-labeled biotinylated HMs. All experiments and controls were conducted in duplicates and all cultures were made up to 5 ml with fresh Mø-SFM and placed at 37°C in a 5% CO_2 humidified atmosphere for 1 h.

Cell Pattern Staining. Following all the magnetic patterning experiments above, cell patterns were visualized by removing the media and staining the cells with Coomassie Blue stain [5.35% (w/v) in 45% methanol:10% acetic acid] for 10 mins. Thereafter, cells were treated with

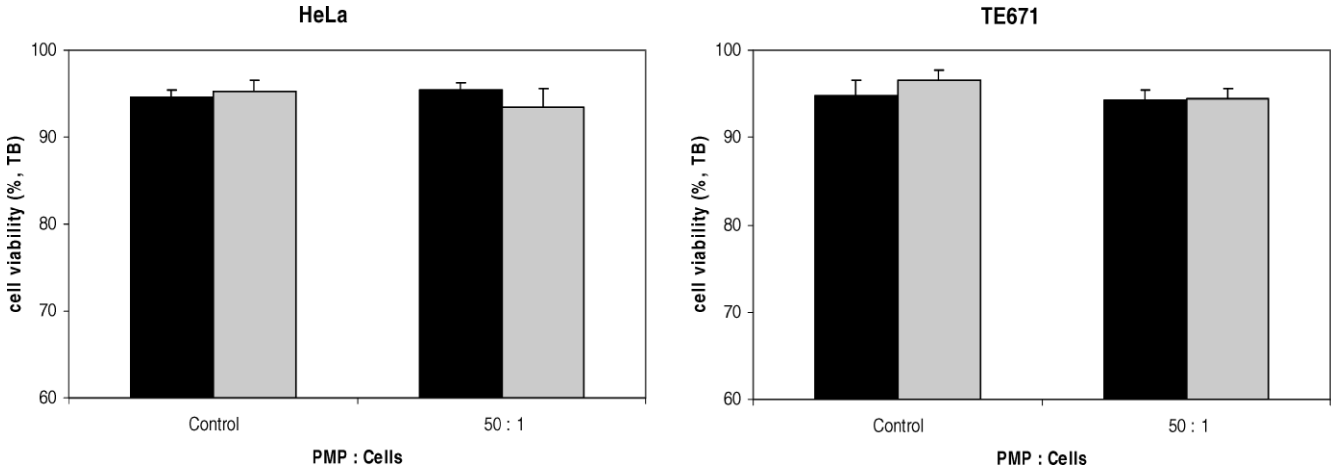

Figure 2. Effect of magnetic labeling on cell viability. Magnetically labeled HeLa and TE671 cells were seeded at a density of 0.01×10^6 cells/well and cultured in the absence (black bars) or presence (grey bars) of a magnetic field. At 72 h, cell viability was assessed using the trypan blue (TB) exclusion assay. Control cells were biotinylated, but not labeled with streptavidin paramagnetic particles (PMP). Values represent the mean ± SE of 2 experiments, each performed in triplicate.

several changes of destain solution (10% methanol:14% acetic acid).

Transmission Electron Microscopy. Magnetically labeled HeLa and TE671 cells were cultured for two days, while the labeled HMs were incubated for 1 h. Cells were washed three times with 0.9% saline. Then, cells were fixed in 4% glutaraldehyde/0.1 M PIPES buffer pH 7.2 containing 3 mM $CaCl_2$ for 1 h at 4°C. Cells were scraped using a cell scraper and subsequently washed three times in PIPES buffer. Following postfixation in 1% osmium tetroxide and bulk staining in uranyl acetate, cells were dehydrated in graded solutions of ethanol (70%, 95%, and 100%) and 100% acetonitrile. Over the following four days, samples were gradually infiltrated with Quetol 651 resin. Thin sections (50–70 nm) were cut using a Leica Ultracut UCT and mounted on bare 300 mesh copper grids. Sections were stained with uranyl acetate and lead citrate for 3 mins each and viewed in a transmission electron microscope (FEI-Phillips CM100) operated at 80 kV in bright field mode.

Results

Cell Viability Studies. In order to examine the effect of magnetic labeling on cell viability, magnetically labeled HeLa and TE671 cells were cultured in the absence or presence of a weak magnetic field for up to 72 h. The trypan blue exclusion assay showed that there was no significant difference in the viability of both cell lines between labeled and non-labeled cells (Fig. 2). Furthermore, the presence of the magnetic field alone was not observed to affect the cell viability in the control sample.

Magnetic Cell Patterning. Figure 3 shows an example of the results of the magnetic cell patterning experiment. Figure 3A-I shows the accurate patterning of magnetically labeled HeLa cells into a distinct star pattern in the area overlying the magnet. In the first control (magnetically labeled cells cultured in the absence of the star-shaped magnet; Fig. 3A-II), a random distribution of cells over the culture dish is apparent. In the second control (biotinylated HeLa cells without magnetic labeling cultured in the presence of magnet; Fig. 3A-III) and third control (non-biotinylated and streptavidin paramagnetic particles-treated cells cultured in the presence of magnet; Fig3A-IV), cells were mainly distributed randomly over the culture dish. However, interestingly, a faint star shape was visible. The cell patterning results for the TE671 cells were similar to that of the HeLa cells (Fig. 3B).

The magnetic field of the star-shaped template (Fig. 3C-I) was mapped using iron filings as shown in Figure 3C-II. The striated pattern that formed over the star-shaped template showed that the magnetic field across its surface was made up of a regular series of field maxima and minima, with the iron filings concentrated on the field maxima. In Figure 3C-III, a plate of magnetically labeled TE671 cells (1×10^6 seeding density) was stained after 3 h incubation with a magnetic star shape underneath the plate. The adhesion of the cells was observed to follow the series of magnetic field maxima.

In the co-culturing of HeLa and TE671 cells, on introduction of the magnetically labeled cells to the dish, they formed rectangular outlines (Fig. 4A), following the magnetic field maxima of the Nd-Fe-B magnet. Since the TE671 cells were seeded and cultured a day longer than the HeLa cells, the TE671 pattern was denser than the HeLa pattern (Fig. 4B). The magnetic field of the Nd-Fe-B magnet used (Fig. 4C) was profiled by using iron filings and the magnetic field profile can be seen in Figure 4D. As expected, the iron filings were concentrated on the field maxima.

As seen previously with the HeLa and TE671 patterning results, the magnetically labeled HMs were attracted by the magnetic shape and formed the star shapes,

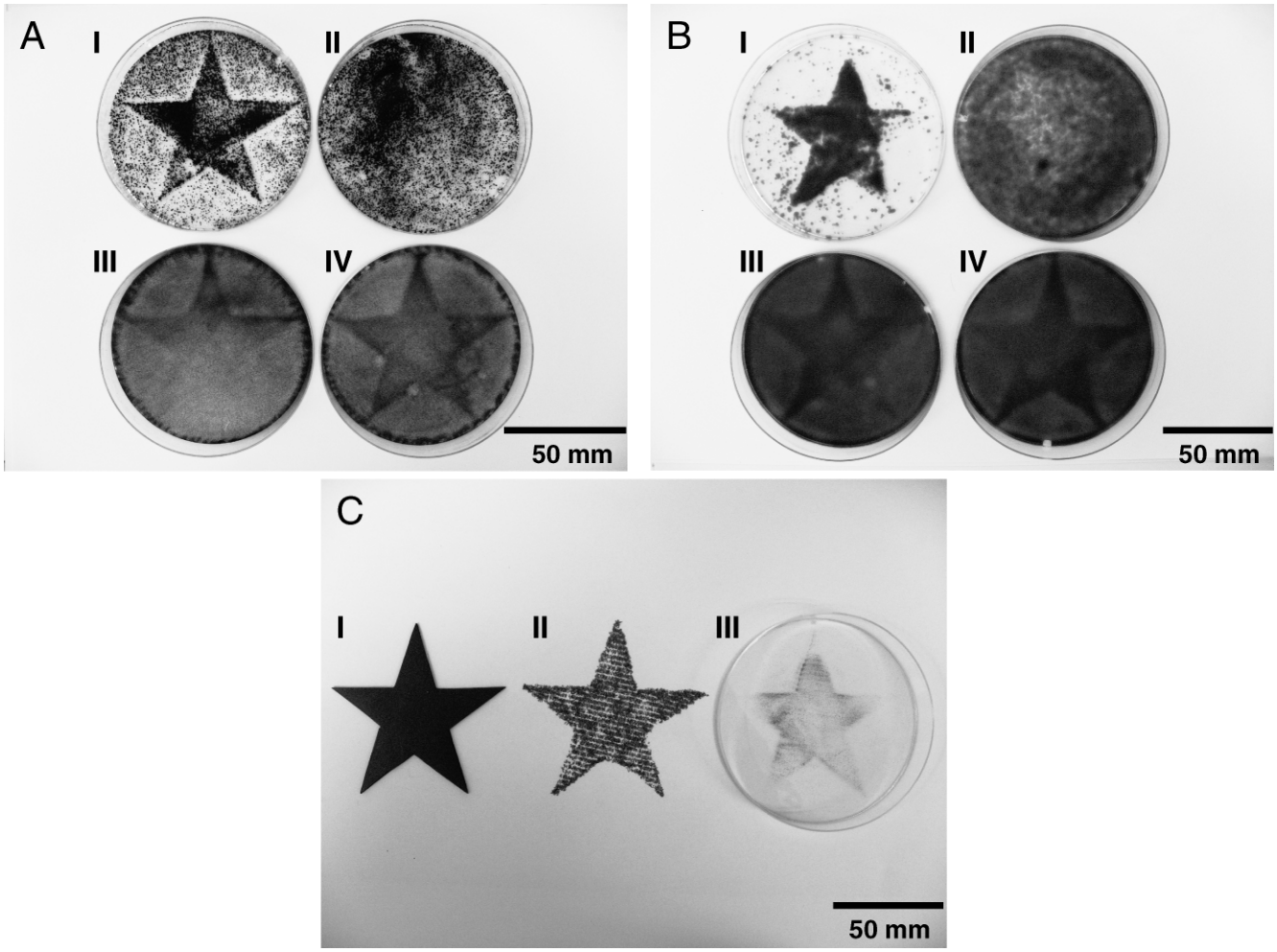

Figure 3. Magnetic cell patterning of biotinylated HeLa and TE671 cells labeled with streptavidin paramagnetic particles **A:** Patterning of magnetically labeled HeLa cells. **A-I:** Magnetically labeled HeLa cells were successfully patterned by the star-shaped magnetic template. **A-II:** Magnetically labeled HeLa cells were not patterned in the absence of the magnetic template. **A-III:** Non-labeled biotinylated HeLa cells were patterned unsuccessfully by the magnetic template. **A-IV:** Non-biotinylated HeLa cells were not labeled successfully and not patterned by the magnetic template. **B:** Patterning of magnetically labeled TE671 cells. **B-I:** Magnetically labeled TE671 cells were successfully patterned by the star-shaped magnetic template. **B-II:** Magnetically labeled TE671 cells were not patterned in the absence of the magnetic template. **B-III:** Non-labeled biotinylated TE671 cells were patterned unsuccessfully by the magnetic template. **B-IV:** Non-biotinylated TE671 cells were not labeled successfully and not patterned by the magnetic template. **C:** Magnetic field profile for cell patterning. **C-I:** Original magnetic template used to pattern HeLa and TE671 cells. **C-II:** Magnetic field profile of the star template used, as visualized by using iron filings to locate magnetic field maxima. **C-III:** Magnetically labeled TE671 cells were targeted by the magnetic field and adhered according to the field maxima of the star template.

as visualized by Coomassie blue staining (Fig. 5A). In the first control, the labeled HMs formed a random distribution on the wells (Fig. 5B). The non-labeled biotinylated HMs were not patterned by the magnetic fields in the second control, as shown in Figure 5C. Similarly, the magnetic field of the template (Fig. 5D) used was mapped using iron filings as shown in Figure 5E.

Transmission Electron Microscopy. The structure of streptavidin paramagnetic particles was examined by transmission electron microscopy. On holey carbon-coated 300 mesh copper grids, the particles were variable in size and showed an irregular shape (Fig. 6A). At higher magnification, it was evident that the paramagnetic particles were composed of aggregates of smaller nanoparticles (Fig. 6B).

After the biotinylated HeLa and TE671 cells were labeled with streptavidin paramagnetic particles and cultured for two days, the particles were observed to be bound to the cell surface (Fig. 7A and C). They were surrounded by membrane ruffles or bound to the plasma membrane, maybe in the process of cellular uptake. A number of paramagnetic particles were also located inside the cells (Fig. 7B and D). The particles were loosely packed in phagolysosomes, but were also seen more densely packed in smaller endosomal compartments. A similar result was found after incubation of the magnetically labeled HMs for 1 h. Some paramagnetic particles were found bound to the

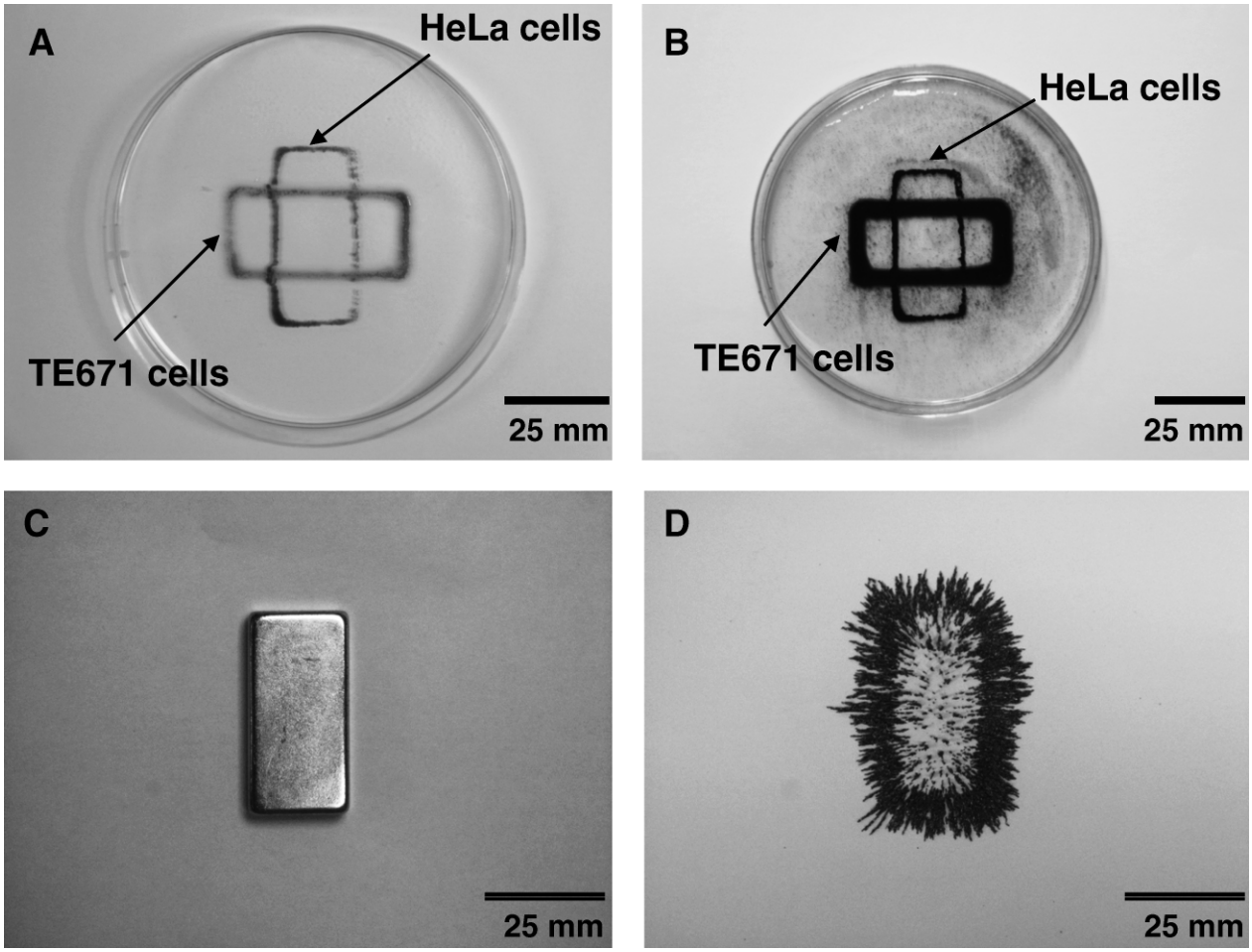

Figure 4. Magnetically controlled co-culture of HeLa and TE671 cells. **A:** The image of the culture dish before Coomassie blue staining. The horizontal rectangular pattern corresponds to magnetically labeled TE671 cells. The vertical rectangular pattern corresponds to magnetically labeled HeLa cells. **B:** The image of the culture dish after Coomassie blue staining. Since the TE671 cells have been cultured for a day longer than the HeLa cells, the TE671 cell pattern is denser than the HeLa cell pattern. **C:** Picture of the Nd-Fe-B magnet used. **D:** Magnetic field profile of the Nd-Fe-B magnet used, as visualized by using iron filings to locate magnetic field maxima.

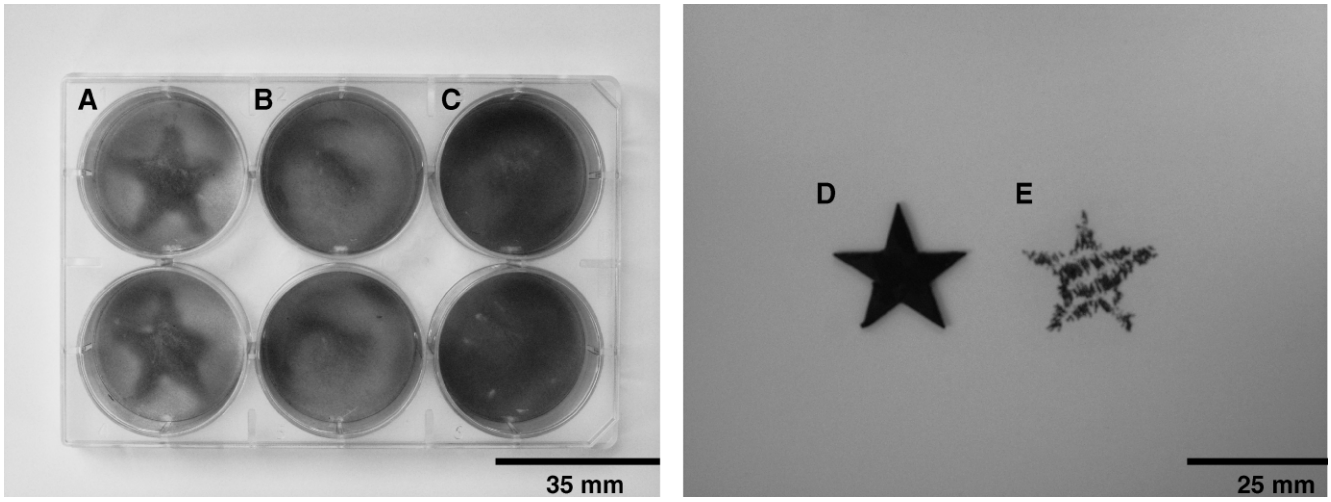

Figure 5. Magnetic cell patterning of biotinylated human monocytes (HMs) labeled with streptavidin paramagnetic particles. **A:** Magnetically labeled HMs were successfully patterned by the star-shaped magnetic template. **B:** Magnetically labeled HMs were not patterned in the absence of the magnetic template. **C:** The non-labeled biotinylated HMs were patterned unsuccessfully by the magnetic template. **D:** Original magnetic template used to pattern HMs. **E:** Magnetic field profile of the magnetic star template used, as visualized by using iron filings to locate magnetic field maxima.

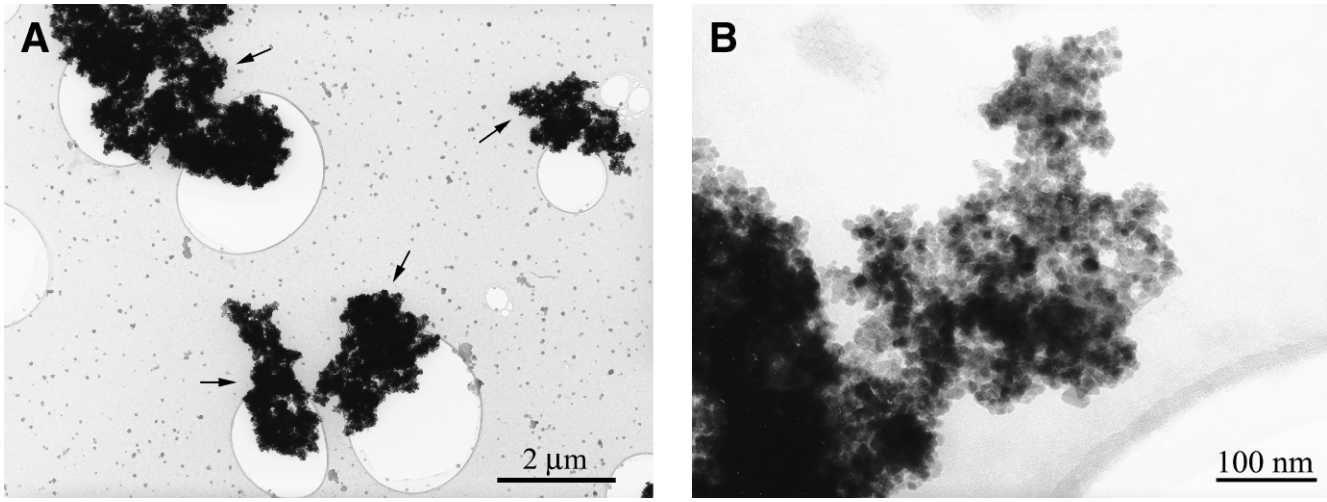

Figure 6. Transmission electron microscopy images of streptavidin paramagnetic particles on holey carbon-coated 300 mesh copper grids. **A:** Streptavidin paramagnetic particles at lower magnification. Arrows point to individual particles. **B:** Streptavidin paramagnetic particles at higher magnification, the aggregate structure of the particles is more evident.

plasma membrane (Fig. 7E), but numerous particles were located inside the cells in large phagolysosomes (Fig. 7F).

Discussion

Our results showed that magnetically labeled mammalian cells could be patterned accurately using the methodology described above. To use this method in biological or clinical applications, it is important that its toxicity is assessed. Cell viability studies showed that there was no cytotoxic effect arising from the magnetic labeling or the exposure to weak magnetic fields in these experiments. Cell proliferation was not observed to be significantly affected by the labeling. In Figure 3A-II and 3B-II, the labeled HeLa and TE671 cells respectively were able to grow to confluence after 72 h. Moreover, as shown in Figure 3B-I and 3C-III, the labeled TE671 cells were able to proliferate and form the star shape, even as they were being targeted by the magnet underneath the culture dish. However, further studies are required to examine the more subtle effects of non-specific magnetic labeling on cellular functions such as cell-cell signaling.

The advancement made by this new methodology for magnetic cell patterning is that it can be applied to pattern a range of mammalian cell types. This may be realized as all mammalian cells express cell membrane proteins, which can be biotinylated and subsequently labeled with streptavidin paramagnetic particles. As a demonstration of the adaptive application of this technique, three different cell types were used in the patterning experiments: HeLa cells, which were derived from cervical cancer cells and are epithelial in nature; TE671, a rhabdomyosarcoma cell line more akin to muscle cells (20); and HMs derived from human blood. HeLa and TE671 are cell lines, which proliferate in culture; whereas HMs are primary cells, which do not proliferate, but will differentiate in culture to form macrophages within 5–7 days.

This two-step method provides a quick way of magnetically labeling cells. The biotinylation step can be carried out for either adherent cells or cells in suspension, as shown by the biotinylation of the adherent HeLa and TE671 cells and the suspended HMs. Without this step, the streptavidin paramagnetic particles would not be able to bind to the cells immediately. This was observed in the control experiment with non-biotinylated cells. Without the biotin tag, the cells were not labeled and they were not patterned by the magnetic fields. Therefore it is evident that cell labeling was due to the specific biotin-streptavidin interaction rather than non-specific interactions of streptavidin with cell surface components. As this technique involves rapid affinity-based attachment of paramagnetic particles rather than cellular uptake of magnetic materials, it could potentially be scaled up. Any amount of cells could be biotinylated *in situ* or in suspension and then labeled with streptavidin paramagnetic particles, without the need for internalization, which is variable between different cell types. For example, mouse fibroblasts and human umbilical vein endothelial cells were cultured with MCLs for 4 h and 24 h respectively for internalization (9). Moreover, this technique creates the possibility of varying the degree of magnetic labeling, as different amounts of streptavidin paramagnetic particles can be added to the biotinylated cells. Current labeling methods are limited in varying the degree of labeling, as cellular uptake of magnetic materials cannot be actively controlled.

The application of the magnetic field ensured directed cell adhesion with an appreciable degree of precision, as shown in Figures 3C-III and 4A. These patterning results showed that the majority of the magnetically labeled cells were targeted by the field maxima and adhered accordingly. Interestingly, the resulting cell distributions in the star

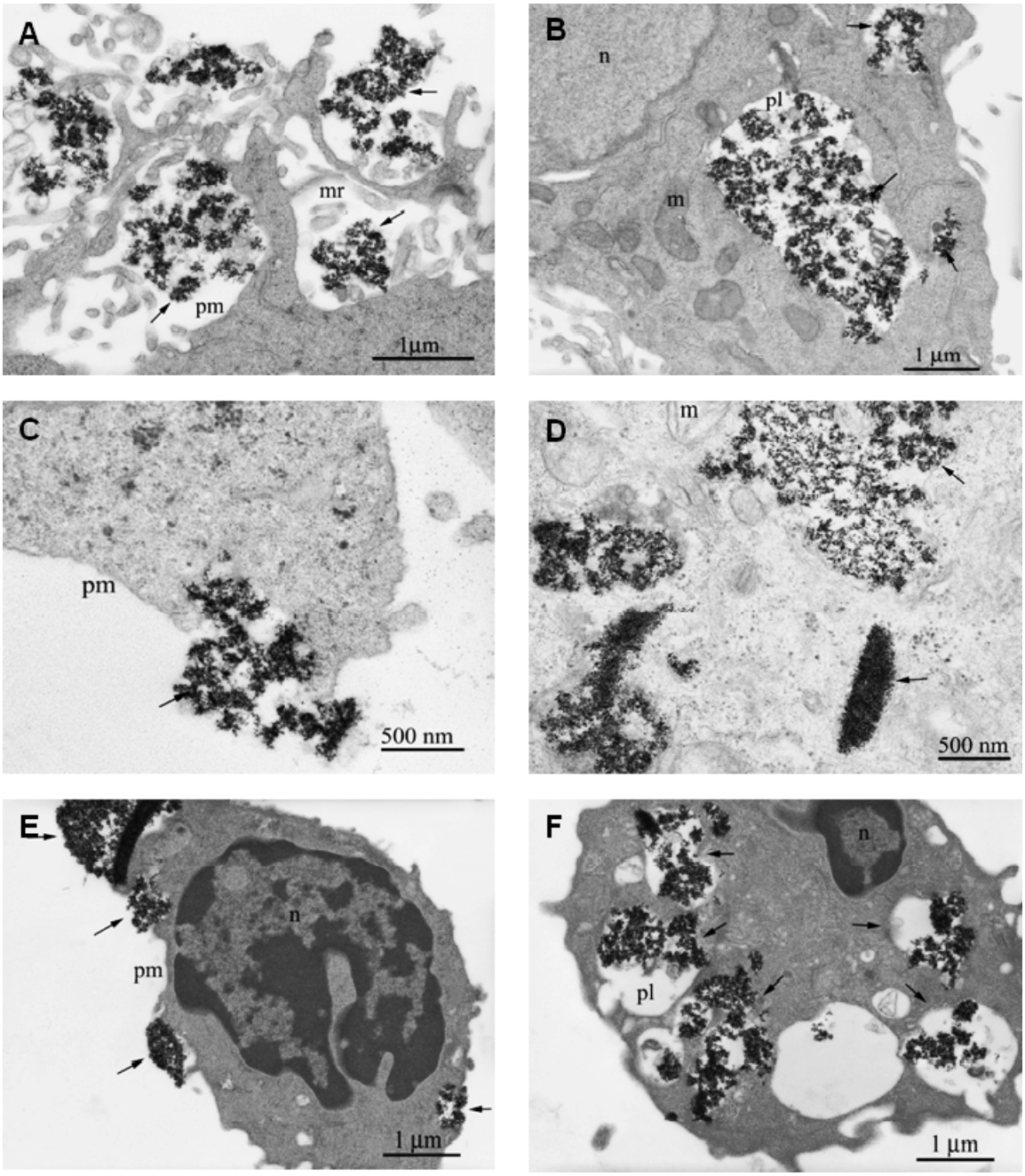

Figure 7. Transmission electron microscopy images of biotinylated cells labeled with streptavidin paramagnetic particles. **A–B:** Paramagnetic particles on the surface and inside HeLa cells, respectively, after two days of culture. **C–D:** Paramagnetic particles on the surface and inside TE671 cells, respectively, after two days of culture. **E–F:** Paramagnetic particles on the surface and inside human monocytes, respectively, after 1 h of incubation. Letters denote: n, nucleus; pm, plasma membrane; pl, phagolysosome; m, mitochondria; mr, membrane ruffles; arrows point at streptavidin paramagnetic particles.

patterns (Fig. 3A-I and 3B-I), after 72 h of culture, did not reflect the striated pattern of magnetic field maxima and minima, as profiled by the iron filings (Fig. 3C-II). One possible reason is that magnetic targeting might be more effective for cells suspended in solution than for cells that are adherent. In addition, cell division will successively dilute the degree of magnetic labeling and weaken the magnetic targeting. For the non-proliferating HMs, the initial cell seeding density was too high for the field maxima effect to be observed, as most of the labeled HMs were attracted by the magnetic field and adhered on top of the star shape. The field gradient was not sufficiently strong to selectively locate the HMs on top of the field maxima. Therefore, patterning of magnetically labeled cells is dependent on strength of magnetic field maxima and cell seeding density.

The cause of the faint star shape observed in two of the controls (Fig. 3A-III, 3A-IV, 3B-III and 3B-IV), grown over the magnetic template, is not certain. It may reflect a very weak effect of magnetic field on these cells. Mammalian cells are charged (21) and naturally possess iron-containing compounds, amongst them ferritin. The interactions between the surface charge on cells, iron-containing intracellular entities and the weak magnetic field might cause a subtle patterning of cells and lead to a higher cell concentration in the vicinity of the magnetic field at confluence. It has also been reported that non-labeled mouse osteoblast cells can be trapped using a modulated magnetic field with maximum strength of 1 T (22). To enhance this effect, paramagnetic materials were added to the cell culture medium. Since DMEM contains iron ions that are magnetic, the presence of low magnetic field underneath the culture dish might cause a weak trapping effect on the non-labeled HeLa and TE671 cells.

Transmission electron microscopy showed that the magnetically labeled HeLa and TE671 cells had internalized some of the streptavidin paramagnetic particles after two days of culture. The uptake may have occurred, as the particles were bound to the cell membranes, thereby promoting the uptake process. Cellular uptake after magnetic labeling could affect particle structure, which may have implications for long-term magnetic cell patterning experiments. The magnetically labeled HMs also took up a significant quantity of the paramagnetic particles, even after only 1 h of incubation. This is not surprising, as HMs are highly phagocytic and have been shown to take up superparamagnetic particles (23). The cellular uptake of paramagnetic particles did not adversely affect cell patterning for the HeLa, TE671 cells and HMs. Other evidence has shown that magnetic manipulation of cells labeled by either internalizing magnetic particles (9) or having particles bound to cell membrane (14) was similarly effective. Furthermore, the uptake of the paramagnetic particles did not affect the viability of both the HeLa and TE671 cells, as shown by the cell viability studies.

It has been shown in these experiments that weak magnets can be used to pattern magnetically labeled cells with a significant degree of precision, after adequately labeling the cells with paramagnetic particles. The maximum magnetic field strength on the surface of the culture dish was measured by using a magnetometer probe (DC Gaussmeter Model 1 by AlphaLab Inc.) and the corresponding values for the Bisiflex II magnetic sheeting, adhesive backed magnetic rubber sheet and the Nd-Fe-B magnet were 0.003 T, 0.008 T and 0.3 T, respectively. Although the magnetic field strengths of the first two materials were two orders of magnitude weaker than the Nd-Fe-B magnet, the cell patterns achieved were well-defined. The malleability of these magnetic materials makes it more feasible to use them for three-dimensional magnetic patterning in the future.

Magnetic cell patterning can provide a substrate-independent method of positioning cells, without the need to fabricate specialized surfaces, such as in soft lithography, or to develop specific equipment, such as the adapted inkjet printer for cell printing. Magnetically labeled cells can be seeded onto any substrate and magnets can then be used to pattern the cells. In this work, magnetically labeled HeLa and TE671 cells were sequentially manipulated using a Nd-Fe-B magnet and co-cultured on the same culture dish with a significant degree of control and precision. The patterning of the labeled HeLa cells on top of the labeled TE671 cells was observed in the intersections of the two rectangular outlines and the layering of different cell types could give rise to three dimensional cellular structures. This can be applied in cell assembly for tissue engineering, as tissues often consist of three dimensional constructs of various cell types. Various artificial tissue structures have been fabricated using similar technologies (14, 24).

Magnetic cell labeling also offers the possibility of non-invasive visualization of cells by MRI after implantation *in vivo* (25). It is thus feasible to track and monitor the implanted labeled cells and study their proliferation and migration *in vivo*. Furthermore, it offers the potential of utilizing external magnetic fields to guide magnetically labeled stem cells *in vivo* in stem cell related therapies (26). Since HMs have been shown to migrate into tumors, magnetically labeled HMs might be used as vehicles for gene therapy with magnetic targeting for enhancing uptake and therapeutic efficacy (27). Magnetically labeled cells could also be used for mechano-induction studies. Hughes *et al.* (28) were able to show that magnetically labeled human osteoblast cells could be mechanically stimulated by external magnetic fields.

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