Electronic Supporting Information

Surface-modified magnetic human cells for scaffold-free tissue engineering

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Experimental details

Chemicals and materials

All chemicals used in this study were purchased from Sigma-Aldrich unless noted, were of analytical grade and used as received. Milli-Q water was used throughout.

Synthesis of MNPs

MNPs were synthesized by mixing 2.0 mL of 1 M FeCl₃ and 0.5 mL of 2 M FeCl₂ aqueous solutions and stirred vigorously, then 50 mL of 0.5 M aqueous NaOH was added dropwise. This resulted in the formation of iron oxide precipitate. The precipitate was then separated using a magnet and washed extensively with water until the supernatant reached pH 7. To stabilize the MNPs with the cationic polyelectrolyte, 1 mL of the nanoparticles was mixed with 10 mL aqueous poly(allylamine) hydrochloride (PAH) (10 mg mL⁻¹), the mixture was sonicated for 10 min (30% power, Bandelin Sonopuls sonifier) and then separated by centrifugation, and washed with Milli-Q water five times. MNPs were filtered using 220 nm pore size syringe filters (Millipore), which removed larger aggregates and sterilized the suspension.

Cell Cultures

A549 cells were cultured in plastic cell culture plates (growth area 25 cm²) at 37 °C in 5 % CO₂ humidified atmosphere using Dulbecco’s modified Eagle media (DMEM) (Invitrogen) supplemented with 10% heat-inactivated bovine serum, 2 mM L-glutamine, and penicillin/streptomycin (100 U mL⁻¹/100 µg mL⁻¹). Human skin fibroblasts (HSF) were cultured in plastic cell culture plates (growth area 25 cm²) at 37 °C in 5 % CO₂ humidified atmosphere using Minimal Essential Medium
Alpha modification (α-MEM) (Gibco) supplemented with 10% heat-inactivated bovine serum, 2 mM L-glutamine, and penicillin/streptomycin (100 U mL⁻¹/100 μg mL⁻¹). Cells were grown to 80-90% confluency and then washed with Dulbecco’s phosphate buffered saline (DPBS) and detached from the wells using 0.25% trypsin–EDTA.

**Functionalization of A 549 and HSF cells with MNPs**

The appropriate concentration of MNPs was determined empirically in preliminary experiments. 1 mL of the sterile MNPs suspension (0.05 mg mL⁻¹) in 0.15 M NaCl was added to cells (10⁶ mL⁻¹) in DPBS. After 3 min incubation during gentle vortexing, the cells were separated from the remaining SPIONs by centrifugation (1 rpm) and washed twice with DPBS. Control samples were subjected to the same procedure, with pure 0.15 M NaCl instead of MNPs dissolved in 0.15 M NaCl.

**Scaffold-free tissue engineering using magnetic cells**

We employed NdFeB cylindrical rod-shaped magnets (E-magnets, UK), having 3 mm diameter, 4 mm length and pull value 0.27 kg.

*First layer:* MNPs-coated HSF (0.3 x 10⁶ cells in each culture wells) were seeded in 6-wells culture plate, then we deposited 3 mm NdFeB cylindrical magnets below the each culture well. The cells were incubated for 24 hours, then the wells were flushed with DPBS to remove the nonattached cells.

*Second layer:* The procedure was repeated using MNPs-coated A549 cells (0.5 x 10⁶ cells in each culture wells). After incubating the cells for 24 hours, the round-shaped multicellular clusters

**Histology.** The clusters were fixed with 10% buffered formaldehyde for 1 hour, then embedded in paraffin blocks and thin-sectioned using a microtome. The paraffin-embedded thin sections (4-5 μm) were dried, deparaffinized, rehydrated and stained using hematoxylin-eosin dyes. Real lung tissue histological samples were kindly provided by Dr. R.A Dzamukov.

**Viability Tests**
The viability of MNPs-coated cells and intact cells was determined using ethidium bromide/acridin orange viability stain. The samples of intact and MNPs-functionalised cells were stained 24 hours after magnetisation (intact cells were not magnetically-modified). Fluorescence microscopy was used to estimate the viability in the stained samples. The adhesion properties and cell index were tested using Real Time Xcelligence System (Roche Applied Science). 5 000 cells in 200 μl medium were introduced in each well of 12-wells plate (E-plate). The cell growth was constantly monitored for 24 hours using the xCELLigence System software.

**Characterization Techniques**

Hydrodynamic diameters and zeta-potentials of MNPs and cells were measured using a Malvern Zetasizer Nano ZS instrument and standard U-shaped cells either in water or in HEPES buffer. Optical and fluorescence microscopy images were obtained using a Zeiss AxioScope A1 microscope equipped with an AxioCam MRc5 CCD camera. Cell culture wells were examined using a Zeiss Observer Z1 inverted microscope equipped with an AxioCam MRc5 CCD camera. TEM images of the thin-sectioned SPION-functionalized cells were obtained using a JEOL 1200 EX microscope operating at 80 kV. The cells were fixed with 2.5% glutaraldehyde, gradually dehydrated using a series of ethanol solutions (30, 60, 70, 80, and 100%), embedded into Epon resin, and then thin sections were cut using a LKB ultramicrotome equipped with a diamond knife and mounted on copper grids. The samples were stained with 2% aqueous uranyl acetate and lead citrate.
Additional Figures:

Fig. S1. A typical SEM image of the PAH-coated MNPs used in this study (scale bar 100 nm).

Fig. S2. Optical microscopy images of suspended A 549 cells – (a) – intact and (b) MNPs-coated and HSF cells: (d) – intact and (d) MNPs-coated.

Fig. S3. Optical microscopy images of intact and MNPs-coated A 549 (a, b) and HSF (c,d) cells colonising the substrates 24 hours after the introduction into the nutrient media (note the brown aggregates of MNPs on both types of cells).