Formation of Ordered Cellular Structures via Label-Free Negative Magnetophoresis

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Statement of Purpose: The creation of ordered cellular structures is important for numerous tissue engineering applications. Magnetic assembly is an attractive means of controlling spatial cell positioning; established technologies involve cell manipulation by attachment of magnetic particles to the cell surface or endocytotic internalization, but both can alter cell function and viability. Here we present a novel strategy for the assembly of cells into linear arrangements by negative magnetophoresis using inert, cytocompatible magnetic nanoparticles (ferrofluid). In this approach, the ferrofluid dictates the cellular assembly without relying on cell binding or uptake and without toxicity. The linear cell structures are stable and can be further cultured without ferrofluid, making this an attractive cell assembly technique for regenerative medicine applications.

Methods: Bovine serum albumin (BSA) – passivated Fe_3O_4 nanoparticles (12 nm diameter) were synthesized as described[1] and PEG - passivated ferrofluid was made identically except substituting Dopamine-mPEG550 for BSA. Suspensions of cells, ferrofluid, and cell media were placed into either a fluid chamber formed with two glass microscope slides coated with BSA, separated by 100-μm spacers, or in 35-mm collagen-coated tissue culture dishes. Magnetic fields were applied by passing current through 2.5 inch iron core solenoids placed across from each other to create a magnetic field that was measured by a gaussmeter. For fluorescent microscopy, human umbilical vein endothelial cells (HUVECs) were stained with CellTracker Green CMFDA®. The kinetics of HUVEC chain growth were examined using a digital camera to take pictures every 30 s for 30 min. Cell viability was measured using LIVE/DEAD assay®. The formation of cell chains was also studied using preosteoblastic cells (MC3T3) and human embryonic kidney cells (HEK293) in BSA- and PEG-stabilized ferrofluid, respectively.

Results: HUVECs in ferrofluid were randomly dispersed before application of a magnetic field (Fig 1a). After the magnetic field was applied the cells chained into linear structures oriented along the field direction (Fig 1b). The average chain length grew with increasing time of magnetic field exposure (Fig 1c), which is consistent with diffusion limited cluster aggregation models that predict a power law dependence for effective chain length as a function of time (Fig 1d).[2] The viability of HUVECs in ferrofluid was examined and found to be unaffected by the presence of ferrofluid for 2 hr, remaining >90%. The cells were able to adhere to collagen-coated tissue culture dishes in ferrofluid under magnetic field, and the ferrofluid could then be rinsed away for further culturing of the cell chains (Fig 2). The ability to use this technique was also confirmed with two cell lines, MC3T3 and HEK293, and one other type of biocompatible ferrofluid, PEG-passivated ferrofluid (Fig 3).

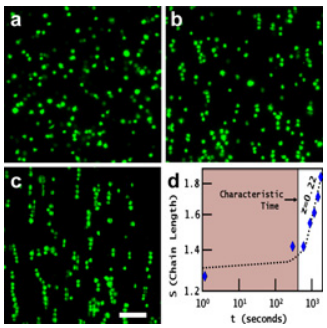

Figure 1. *Kinetics of cell chain formation. (a) HUVECs in absence of magnetic field in BSA-ferrofluid. After magnetic field application, (b) cells align by 30 secs, and (c) cell chains grow in length by 5 mins. Scale bar represents 100 μm. (d) Log-log plot of effective chain length vs. time (◆) following a power law-dependence consistent with general colloidal aggregation models (dotted line).*

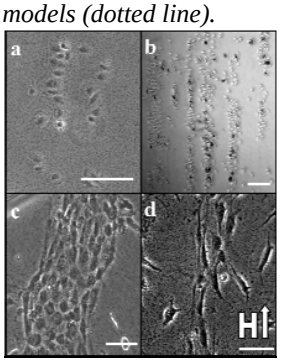

Figure 2. *Chain structures of HUVECs on collagen-coated plates, formed with BSA-ferrofluid (a, b) after 2-hr exposure to a magnetic field. (c, d) HUVEC chain structures incubated overnight without ferrofluid. Magnetic field, H, applied in the direction of the arrow. Scale bars represent 100 μm.*

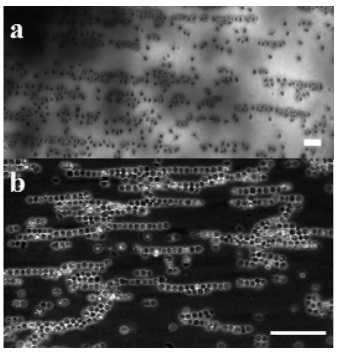

Figure 3. *Linear cell structures formed with (a) MC3T3s in BSA-ferrofluid under 100 Oe field and (b) HEK293s in PEG-ferrofluid under 200 Oe field. Scale bars represent 100 μm.*

Conclusions: We have developed a novel strategy for the linear arrangement of cells using inert, cytocompatible magnetic nanoparticles which shepherd the cells into linear, oriented structures under uniform magnetic fields through negative magnetophoresis. The lengths of the cellular chains were found to depend upon exposure time. The linear cell assemblies are stable after removal of the magnetic field, and the cells are able to adhere to standard tissue culture surfaces and be further cultured without the ferrofluid. Finally, the magnetic nanoparticles are shown to have no cytotoxic effects on the cells. This approach is highly versatile and has been demonstrated to be effective using multiple cell types.

References: [1]Samanta et al. J Mater Chem 2008:18:1204. [2]Cernak et al. Physical Review 2004:70, 03/15/04.

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