Magnetically Assisted Pattering for the Biofabrication of Branched Vascular Structures

Benjamin Whatley [1], Jeffrey Morgan [2], Xuejun Wen [1,3]
[1]Clemson - MUSC Bioengineering Program, Department of Bioengineering, Clemson University, Clemson, SC, 29634
[2]Department of Medical Science and Engineering, Brown University, Providence, RI 02912
[3]Department of Orthopaedic Surgery, Cell Biology & Anatomy, Medical University of South Carolina, Charleston, SC, 29425

Statement of Purpose: There is a high demand for tissue engineered organs, as the shortage of donor organs increases daily.[1] The necessity for tissue engineering strategies is apparent, and research into the regeneration of a working vascular system has been increasingly investigated. However, none of existing technologies have proven successful in the regeneration of a complete vascular network in engineered tissues. We developed a complex form of biofabrication allowing for advanced spatial patterning of multicellular spheroids into branched vessel-like tubule structures using magnetically-assisted patterning. Multicellular spheroids have been shown to be useful in the beginning stages of the reconstruction and engineering of organs, especially blood vessels.[2] It has been shown that endothelial cell spheroids will fuse together when cell-cell contact is encouraged.[3] This fusion promotes the formation of hollow lumen-like tubules which mimic the native vascular structure.[3] Using magnetic patterning to control the temporal location of paramagnetic multicellular microspheres allows the precise patterning of cells to guide cell attachment and encourage cell-cell contact along a CAD based pattern.

Methods: Super-paramagnetic iron oxide nanoparticles were sterilized using gamma irradiation and incorporated into endothelial cells before spheroid formation. A biocompatibility study was employed to identify the highest non-toxic loading of paramagnetic nanoparticles in endothelial cells (Fig. 1). Cells were also stained using a live/dead stain and analyzed using flow cytometry to determine the effect of nanoparticle concentration on cell viability. These magnetic nanoparticle loaded cells were used to make uniform multicellular spheroids using ultra-hydrophilic microwells (Fig. 2A). The micropsheres were analyzed to ensure consistent size uniformity. Then, the cell spheroids were placed on magnetically branched patterns with the hopes of inducing self-assembly of the microspheres. Branched patterns were designed using CAD, and these patterns were cut from a magnet sheet using a CAD controlled cutting device. Uniform size magnetic endothelial cell microspheres were placed in tissue culture wells with magnetic branched patterns at the outside bottoms of the wells. After 1 week in culture, the structures were analyzed using confocal microscopy and microsphere fusion into tubules was investigated.

Results: The highest concentration of paramagnetic nanoparticles that did not affect cell viability was 2.5 mg/mL (Fig. 1). Endothelial cells were loaded with this concentration of paramagnetic nanoparticles and were used to create highly uniform microspheres with a diameter of 250 µm (Fig. 2). The magnetic multicellular spheroids were attracted and patterned directly onto the CAD magnetic branches. The magnetic field generated by a thin magnetic pattern placed outside the culture wells is strong enough to induce the patterning of the free floating super-paramagnetic nanoparticle loaded endothelial cell spheroids (Fig 3).

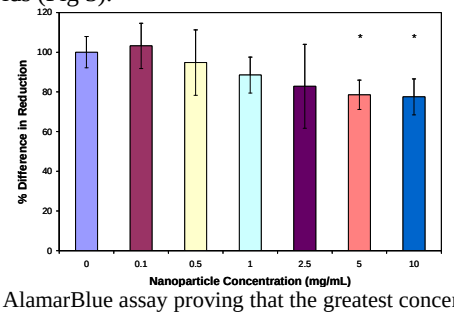

Fig. 1: AlamarBlue assay proving that the greatest concentration of magnetic nanoparticles not affecting cell viability was 2.5 mg/mL (n=6).

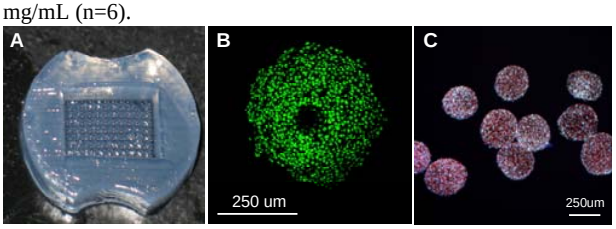

Fig. 2: Creation of uniform microspheres in hydrophilic microwells with paramagnetic nanoparticles encapsulated by endothelial cells (A). Microspheres showed no decrease in cell viability with LIVE/DEAD assay (B). Consistently sized, uniform microspheres were formed (C).

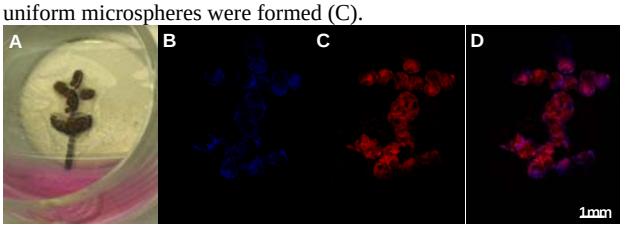

Fig. 3: Paramagnetic microspheres attached on branched magnetic pattern (A). Spatially patterned microspheres after 1 week in culture: nuclei (B), actin (C), and merged image (D).

Conclusions: The use of multicellular spheroids enabled 3D branched tubular formation similar to the native vascular network. It was shown that an optimal concentration of paramagnetic nanoparticles exists that does not significantly affect cell viability. Multicellular microspheres were created with consistent size uniformity while retaining their paramagnetic properties. This technique patterned magnetic multicellular microspheres into a branched structures in an attempt to show that spatial patterning can be controlled. Cell-cell contacts were also promoted which may promote tubular formation into an endothelialized vessel structure.

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