Controlled Release Biomaterials for Directed Embryonic Stem Cell Differentiation

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Statement of Purpose: Stem cell technologies have shown great potential in regenerative medicine applications, yet various limitations have prevented their use clinically, including the inability to induce controllable, homogenous differentiation to a targeted cell type. In the developing embryo, signals that direct differentiation are presented in a temporally and spatially controlled manner. The effects of various molecules on embryonic stem (ES) cell differentiation in vitro can vary depending on the concentration and timing of introduction to cells [1,2]. Thus, techniques for presenting signaling molecules to ES cells in a spatially and temporally controlled manner need to be integrated into differentiation methods. Polymer microspheres have been employed as both an adherent surface for cells and a biomolecular delivery vehicle in cellular transplantation studies [3]. The objective of this research is to incorporate microspheres within the local microenvironment of differentiating three-dimensional clusters of ES cells known as embryoid bodies (EBs) to increase the homogeneity of biomolecular distribution and achieve more homogeneous differentiation.

Methods: Poly(lactic-co-glycolic acid) (50:50, MW 75 kDa) microspheres encapsulating retinoic acid (RA), a potent differentiation factor for ES cells, were fabricated using an oil-in-water emulsion, solvent evaporation technique. Microspheres were analyzed using scanning electron microscopy and particle size analysis (Coulter Multisizer III) and the release characteristics were assessed spectrophotometrically ($OD_{360\ nm}$). Untreated and gelatin coated microspheres were added to both hanging drop and suspension ES cell cultures. Phase microscopy imaging as well as hematoxylin and eosin staining of EB cross-sections were used to assess the incorporation of microspheres within EBs. Confocal imaging (Zeiss LSM 510) has been used to image labeled EBs (CellTracker™ Red, Molecular Probes, Carlsbad, CA).

Results / Discussion: PLGA microspheres displayed regular, spherical morphology with smooth surfaces (Figure 1A), and no noticeable differences were observed between RA encapsulated and blank microspheres. Microsphere efficiencies of up to 77% were achieved and RA encapsulation efficiency was found to be 33.8 ± 5.1%. Particle size analysis revealed the average particle size to be 4-5 μm (Figure 1B). Release study data has shown that RA is released from the degradable microspheres over the course of two weeks, the period critical in determining ES cell fate. Phase contrast microscopy indicated that microspheres were associated with EBs

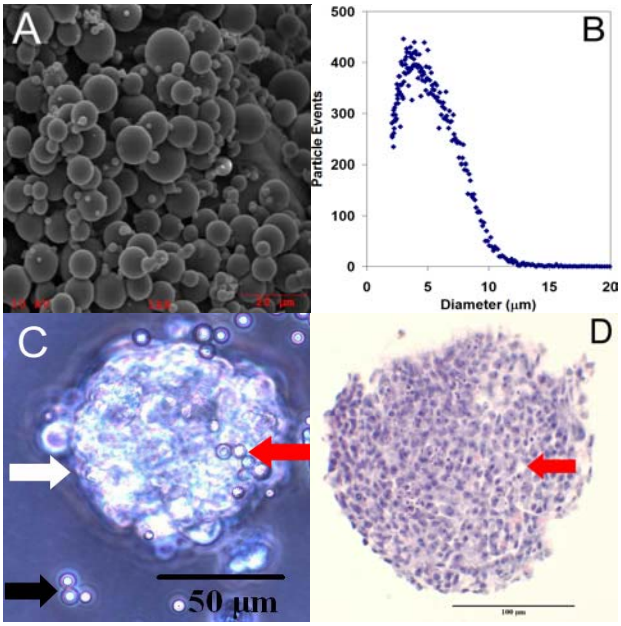

Figure 1. Analysis of microsphere (A) morphology and (B) size distribution. (C) Phase image of EB-microsphere construct. White arrow indicates an EB, black arrow indicates free microspheres, red arrow shows microspheres physically associated with the EB. (D) H&E stained EB, with red arrow highlighting a void.

(Figure 1C), and that gelatin coated microspheres appear to be incorporated at a higher frequency than uncoated microspheres. Hematoxylin and eosin staining of EBs revealed the presence of voids, possibly the result of microspheres that were dissolved during histological processing (Figure 1D). Confocal imaging has revealed the 3-D structure of labeled EBs, and current experiments are focused on imaging fluorescently labeled microspheres within EBs to obtain quantitative spatial information on EB incorporation.

Conclusions: Controlled release microspheres containing differentiation factors can be incorporated within 3D aggregates of ES cells. This provides us with a method to present signaling molecules in a controlled manner within the microenvironment of differentiating ES cells. Using this technique, the effects of differentiation factor distribution on homogeneity of ES cell differentiation can be examined. Future experiments will utilize real-time RT PCR and immuno-staining with flow cytometry to quantitatively assess the effect of controlled RA presentation on ES cell differentiation.

References:

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