Poly(ethylene glycol) microsphere-encapsulated pluripotent stem cells: Control of microsphere size for cardiac differentiation

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Statement of Purpose: Specialized mammalian cells of the cardiac lineage, cardiomyocytes, exhibit the unique ability to beat due to their advanced electrophysiological properties and highly developed electromechanical coupling. Adult cardiomyocytes, however, cannot be readily expanded *in vitro* while maintaining their specialized phenotype.Pluripotent stem cell sources are therefore currently under investigation for applications in regenerative medicine; these cells have the potential to be expanded and then differentiated down thee cardiac lineage to become cardiomyocytes. A major challenge in stem cell engineering is deriving, through expansion and differentiation, a homogeneous population of specialized cells from an initial pluripotent stem cell population. In the case of embryonic stem cells, the standard differentiation methods often begin by hanging-droplet embryoid body (EB) formation, providing control over the number of aggregated stem cells and resulting in round EBs. This technique, however, is not readily scalable and does not allow for control over the cell's microenvironment. Studies using micropatterning techniques[1] and microsphere encapsulation[2] have agreed that control of stem cell EB colony size influences cell fate . This research investigates the differentiation of pluripotent stem cells encapsulated within poly(ethylene glycol)-based hydrogel microspheres, and the effect of microsphere size on stem cell differentiation. Water in oil emulsion and needle extrusion processing methods were investigated for their potential in creating microspheres of desired size with a monodisperse size range.

Methods: Poly(ethylene glycol) diacrylate (PEGDA) microsphere hydrogels were processed using two methods, emulsion and extrusion. In the emulsion method, 10% PEGDA in PBS solutions contained 154 nmole/mL Eosin Y photoinitiator, 5 µL/mL N-vinylpyrrolidone, 1.5 µL/mL triethanolamine. Following published protocols[3], microspheres were produced by emulsifying the aqueous phase in mineral oil containing 5 µL/mL 2-2-dimethoxy-2-phenyl acetophenone in N-vinylpyrrolidone (300 mg/mL) while undergoing simultaneous vortexing and exposure to light from a metal halide lamp source. Microsphere sizes were varied by using different processing conditions such as vortex speed, crosslinking time, and water/oil ratio. The extrusion technique follows published protocols[4] using a Tetronic T1107 30% and PEGDA 12% in PBS solution containing 5 µL/mL 2-2-dimethoxy-2-phenyl acetophenone in N-vinylpyrrolidone (300 mg/mL) which was extruded from a syringe needle and dropped into a 10% Pluronic F127 in PBS bath containing 0.1% (wt/v) Tween 80 and covered by a layer of n-hexane. Spheres were formed upon impact with the bath, and polymerization was achieved by brief exposure to a UV lamp. Extruded microsphere size was controlled by varying extrusion rate and polymer solution viscosity. Cells used were bovine aortic endothelial cells (BAEC) for viability studies, and mouse embryonic stem cells (mESC) for viability and differentiation studies. Cell viability was assessed using live/dead stain imaging with calcein AM and ethidium homodimer. Cardiac differentiation was determined by evaluating percentage of beating spheres as well as staining for cardiac-specific markers alpha-myosin heavy chain and connexin43.

Results: Cells were found to survive the emulsion microsphere processing method via live/dead stain imaging. The emulsion method provided the ability to control average sphere size, although suspensions with a highly polydisperse size range were produced, as shown in Figure 1. The extrusion method produced spheres with monodisperse diameters, although initial studies found that sphere size was above the micron range due to high viscosity of the polymer solution.

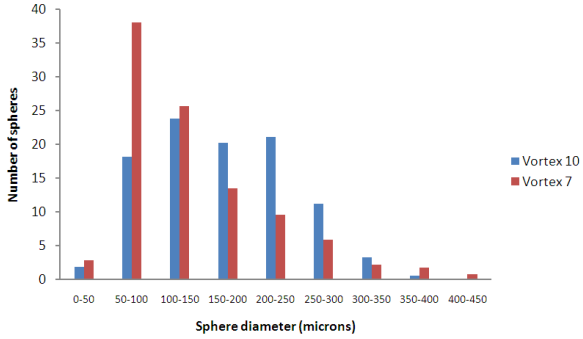

Figure 1. The effect of vortex speed on size distributions of microspheres produced via emulsion

Conclusion: This study investigates two microsphere processing conditions to control stem cell colony size and thus the differentiation fate of pluripotent stem cells. Stem cell encapsulation within hydrogel microspheres is a scalable alternative to the hanging drop method of EB formation. Hydrogel microspheres allow for sophisticated control of EB size. Further work will covalently incorporate into the PEGDA backbone additional components for cell attachment and proteolytic degradation, in addition to signaling molecules to direct cardiac-specific differentiation. In review, microsphere encapsulation technology is a promising technique for stem cell differentiation because it allows for the control of structural characteristics of the cell's microenvironment during the formation of embryoid bodies.

References

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