Engineering of Stem Cell Microenvironments by Incorporation of Microsphere Biomaterials

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Introduction: Pluripotent embryonic stem cells (ESCs) are a potent cell source for a variety of tissue engineering and regenerative medicine applications due to their unique ability to differentiate into the full spectrum of somatic cells constituting the ecto-, endo- and mesoderm germ lineages. The tremendous potency of ESCs also poses a significant challenge to their successful implementation in regenerative therapies – the inability to efficiently differentiate the cells to defined phenotypes in a homogeneous and efficient manner using scalable techniques. Most attempts thus far to direct ESC differentiation *in vitro* have relied on continuous exposure to defined concentrations of soluble factors and/or surface chemistries in 2D to elicit changes in cell fate decisions. However, tissue morphogenesis and cell patterning are dynamic processes that are normally orchestrated in 3D in a precise spatio-temporally controlled manner. Thus, in order to more accurately recapitulate developmental patterning and enhance the homogeneous differentiation of ESCs, the objectives of this work were to 1) engineer microspheres from different biomaterials capable of delivering various morphogens, 2) determine robust and effective means of incorporating microspheres within 3D aggregates of ESCs, and 3) examine the effects of microsphere incorporation and morphogen delivery on ESC differentiation as embryoid bodies (EBs).

Materials and Methods: Microspheres (~1-10 μm diameter) were fabricated from poly(lactic-co-glycolic acid) (PLGA), gelatin or agarose using conventional oil-in-water or water-in-oil emulsion techniques and labeled by physical entrapment or covalent conjugation of fluorescent dye molecules (i.e. CellTracker or Alexa probes). Small molecules, such as retinoic acid, were directly incorporated within PLGA microspheres during formation, whereas proteins of various molecular weights (i.e. bFGF, chymotrypsinogen, BSA) were entrapped within microgels after fabrication. Microspheres were mixed with mouse ESCs (D3) at various ratios during EB formation using rotary orbital culture or forced aggregation techniques in PDMS microwells. Following initial microsphere incorporation, EBs were re-fed every 2 days and maintained in rotary orbital suspension for the duration of culture (up to 14-21 days). At different stages of differentiation, EBs were examined by confocal microscopy analysis in order to assess the abundance and spatial distribution of microspheres within EBs, as well as the release of fluorescently labeled species from the incorporated microspheres. Gene expression and immunohistochemical analysis for pluripotent and differentiated markers were performed to determine the phenotypic effects of different microspheres and microsphere-mediated delivery of morphogens on the differentiation of ESCs in EBs.

Results: Microspheres were stably incorporated and distributed throughout the interior of EBs within the first 24-48 hours of spheroid formation, and microsphere incorporation did not adversely affect the kinetics or size of initial EB formation. Overall, adhesive microspheres and smaller size particles were incorporated more readily than non-adhesive and larger microspheres, respectively, in a dose-dependent manner that could largely be controlled by the microsphere:ESC seeding ratios (1, 2). Microspheres were stably incorporated within EBs over the course of differentiation (up to several weeks), but gradually degraded over time with normal kinetics for PLGA hydrolysis and gelatin enzymatic degradation. Compared to soluble treatment controls, fluorescently-labeled molecules released from incorporated microspheres were more homogeneously distributed throughout the population of cells comprising 3D ESC spheroids. Interestingly, the presence of different microsphere materials within EBs differentially affected gene expression profiles and elicited differences in the spatial expression patterns of phenotypic markers for differentiated cell types. Moreover, morphogen delivery (i.e. retinoic acid) from degradable PLGA microspheres induced significantly different patterns of gene expression and homogeneous differentiation of spatially organized EBs compared to soluble treatment regimens (1).

Conclusions: These results convincingly demonstrate that microsphere materials can be used to directly modulate the 3D microenvironment of ESCs and deliver morphogens in order to direct differentiation more efficiently than conventional soluble treatment techniques. In addition to soluble morphogen factor delivery, microspheres can also be used to present extracellular matrix molecules, cell adhesion receptors and other molecular regulators of stem cell differentiation (3). This approach to engineering stem cell microenvironments represents a novel strategy to apply principles of biomaterials and drug delivery design to the development of stem cell technologies for regenerative medicine and diagnostic applications.

References:

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