Designing Materials to Direct Stem Cell Fate

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Human embryonic stem (**hES**) and induced pluripotent stem (**hiPS**) cells are being studied as potential sources of cells for the treatment of many disorders (e.g. diabetes, spinal cord injury, Parkinson's, leukemia, congestive heart failure). These same cells are also being touted as an ideal source for *ex vivo* tissue engineering or *in situ* regenerative medicine. The successful integration of hES and hiPS cells into such therapies will hinge upon three critical steps: 1) stem cell expansion in number without differentiation (i.e., self-renewal); 2) directed differentiation into a specific cell type or collection of cell types; and, 3) promotion of their functional integration into existing tissue. Precisely controlling each of these steps will be essential to maximize stem cell therapeutic efficacy. However, it is difficult to precisely control the behavior of these cells, since environmental conditions for self-renewal and differentiation are incompletely understood. Over the past decade my group has been studying these critical bottlenecks using various materials platforms, two of which will be addressed in this presentation.

1) Stem Cell Self Renewal

Historically, hES and hiPS cells have been grown in monolayer culture with a feeder layer of mouse cells (i.e., irradiated but viable cells) and/or with conditioned media derived from these feeder cells. Rapid advancement in cell culture techniques has led to the development of chemically-defined media and feeder-free hES cell culture systems that employ animal or human-derived extracellular matrix (ECM) proteins to coat the culture substrata.[1] For chemically-defined media, Matrigel™ is the most prevalent ECM analogue, and is an extraction from Engelbreth–Holm–Swarm mouse sarcomas that contains basement membrane components and numerous growth factors.[2] As Matrigel™ represents a poorly defined substrate for precise stem cell expansion, more recent advances have focused on replacing Matrigel™ and isolated ECM proteins with recombinant proteins, synthetic peptides,[3-5] and/or polymers.[6] Synthetic peptide interfaces have achieved the most promising results, having demonstrated long-term expansion in chemically defined media. However, there is still significant marginal cost associated with these systems, potentially limiting their use in the large scale manufacturing, where a requirement to deliver 10^9 cells per patient is estimated for many stem cell therapies. Therefore, we have developed a synthetic polymer interface for the long-term self-renewal of hES cells in chemically defined media. We cultured hES cells on hydrogel networks of aminopropylmethacrylamide (APMAAm) for over 20 passages in chemically defined mTeSR™1 media and demonstrated pluripotency of multiple hES cell lines with immunostaining and quantitative RT-PCR studies. Results for hES cell proliferation and pluripotency markers were both qualitatively and quantitatively similar to cells cultured on Matrigel™-coated substrates. Mechanistically, it was resolved that bovine serum albumin in the mTeSR™1 media was critical for cell adhesion on APMAAm hydrogel interfaces. This study identified the first long-term culture surface for the self-renewable of hES cells without the preadsorption of biologic coatings (e.g., peptides, proteins, or Matrigel™) in completely chemically defined media. We envision this system to minimize cost, bring the practice in line with current cell culture methods, and aid in standardizing the process toward clinical-scale hES cell expansion.

2) Directed Differentiation

Highly regulated signals in the stem cell microenvironment, such as growth factor presentation and concentration, matrix stiffness, and ligand adhesion density have been implicated in modulating stem cell proliferation and maturation. Therefore, it is desirable to have independent control over both the biochemical and mechanical cues presented to the cell to analyze their relative and combined effects on stem cell function.[7, 8] Accordingly, we have developed synthetic hydrogels to assess the effects of soluble signals, adhesion ligand presentation, and material moduli spanning physiologically relevant ranges (10 to 10,000 Pa) on stem cell function.[4,9] Employing these soft materials, we have demonstrated that the mechanical and biochemical properties of a stem cell microenvironment can be tuned to regulate the self-renewal and differentiation of different stem cells including human embryonic, neural, and mesenchymal stem cells.

Selected References:

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