Cell, Stem cells and Regenerative Medicine

Guiding Stem Cell Differentiation through Matrix Optimization

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Extrinsic factors, such as matrix stiffness and bioactive signaling molecule concentration, play a significant role in stem cell lineage choice and maturation [1-4]. However, the extracellular matrices used to support stem cell differentiation are often chosen based on convenience and initial favorable result without consideration for the ability or ease of further matrix modification in order to better promote differentiation to the desired lineage or tissue type. Rationally designing artificial extracellular matrices to modulate stem cell behavior and better guide lineage choice and maturation will lead to improvements in derivation protocol efficiency and tissue formation. These improvements will increase in the number of stem cell therapy and tissue engineering treatments entering the clinic and potentially improve their efficacy.

Small changes in environmental conditions can have a large effect on stem cell behavior and differentiation [1, 2, 5]. This would suggest that each desired lineage and tissue engineering application may require its own optimized matrix, which is a major undertaking. These optimizations are made more difficult due to the lack of knowledge about how many biomaterial properties and the concentrations of incorporated bioactive signaling molecules affect stem cell behavior. This is further complicated by the fact that cellular responses can change between species [6] and cellular differentiation states [7,8], an indication that multiple or dynamic matrices may need to be optimized in order to shepherd a cell from a pluripotent state to a terminal differentiation. To address these issues and work toward optimized materials capable of guiding stem cells to desired lineages and tissues, systematic approaches to matrix design need to be more widely adapted. Combinatorial methods, which have long been used in the pharmaceutical industry, offer the potential to reduce matrix optimization times through systematic study and are being applied to examine stem cell-biomaterial interactions.

Although many combinatorial methods exist, few are suitable for application to three-dimensional culture. Even fewer have been applied to biomaterial development [1,9,10] of these methods, the continuous gradient approach offers a straight forward way to systematically optimize biomaterials for stem cell culture. As every possible test condition from the range is present within the sample, alterations in stem cell response to changes in the test condition can be quickly identified. The test range can then be quickly modulated to study the region of interest in greater depth. As the exact nature of the cellular response and the range in which it occurs are often not known for stem cells prior to the start of the study, the ability to shift the range of study quickly is a significant advantage. A recent study examining the effects of IKVAV, a bioactive peptide isolated from laminin, concentration on the neural differentiation of mouse embryonic stem cells in two- and three-dimensional culture noted a significant shift in the concentration range of interest and proceeded to half the IKVAV concentration for the three-dimensional studies to provide greater resolution on the new area of interest [1].

The continuous gradient hydrogel platform utilized in that study provides easy transition between two- and three-dimensional cultures of stem cells with minimal changes to the fabrication of the gradient sample. However, significant changes to the extracellular environment presented to the cell exist between two-and three-dimensional culture due to changes in diffusion, concentration and persistence of cytokines and tethered bioactive factors between the culture types. These differences in cellular environment can lead to significant changes in stem cell response, as they did in the study by Yang and colleagues [1]. Both, two-and three-dimensional culture are useful in stem cell culture and need to be studied in greater detail to understand how the differences in the cellular environment they provide lead to changes in cellular behavior. Two-dimensional culture limits matrix contact with the cell to one plane, similar to traditional cell culture. This environment could prove favorable for lineage derivation protocols as it allows for easy isolation of the cells for sorting and further processing. Three dimensional cultures surrounds the cell, better mimicking the in vivo environment, which will be necessary for de novo tissue formation and potentially then maturation of certain cell types.

Crosstalk between cells cultured in different conditions can significantly affect cellular behavior. The continuous gradient hydrogel platform offers the ability to modulate cellular crosstalk between sample conditions. Samples can be cultured whole, allowing access to cytokines secreted by cells in all positions within the gradient, or sectioned and cultured in isolation, which allow access to cytokines secreted by cells in nearly similar conditions. A study of human mesenchymal stem cell lineage choice found a significant difference in osteogenic and adipogenic differentiation when available cellular crosstalk was modulated in this

way [11]. Free access to cytokines across the gradient was found to favor adipogenic differentiation, while limited cytokine access promoted osteogenic differentiation. This study indicates that secreted cytokine access should be a significant design consideration in these studies as it has significant implications on experimental outcomes with stem cells.

Our biological understanding of stem cell biology has advanced greatly over the last few decades. Often it is hard to remember that the extracellular matrix was thought of as biologically inert not that long ago. As such, many of the biological considerations that artificial extracellular matrices try to address are relatively new and matrix design considerations are changing rapidly. The systematic approaches discussed here are powerful tools, which will further speed this progression. They will highlight important matrix design considerations and illustrate relationships between cells and matrices previous unknown and unconsidered as they add to the base of knowledge. As our understanding of both stem cell biology and stem cell-matrix interactions grow, so will our success at manipulating stem cells toward the lofty promises of regenerative medicine and tissue engineering, cures for what ails so many of us.

References

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