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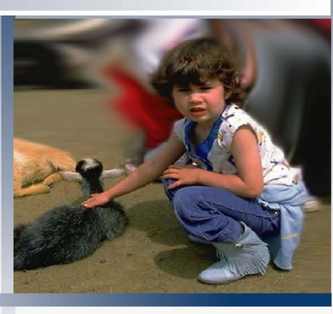
Laboratory of Comparative Genomics

Directed Differentiation of Porcine Mesenchymal Stem Cells in Three Dimensional Matrices



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Abstract

The stem cell niche represents a heterogeneous environment supporting mesenchymal stem cell (MSC) self-renewal and differentiation. However, the mechanisms with which niche signals maintain self-renewal or direct differentiation remain unclear. The objective of this study was to develop an approach to visualize and identify niche signals that maintain MSC self-renewal and differentiation. A culture system was developed where laser tweezers manipulate and encapsulate individual cells into specific positions within a three-dimensional (3D) scaffold and signaling kinetics are regulated by microfluidic devices, controlled release microspheres, or other niche cells. This system was designed to control individual cells and the signals they receive. To validate laser tweezer manipulation, we employed the U937 cell line prior to MSCs because they easily differentiate into macrophages by diffusible small molecules (phorbol 12-myrisate 13-acetate; PMA). Poly (ethylene glycol) diacrylate (PEGDA) and alginate were analyzed as a scaffold for 3D culture systems. PEGDA is an easily modified synthetic matrix that is rapidly photopolymerizable in a controlled manner and permits signal and waste diffusion. Alginate is a natural polymer that facilitates nutrient and waste diffusion and is rapidly polymerized by Ca2+ ions. U937 cells and MSCs were successfully manipulated by laser tweezers into homotypic 4x4 arrays and encapsulated in the 3D scaffolds. This approach was also successful in forming heterotypic arrays of U937 and MSC with various cell types. Large cell arrays were formed by juxtaposing several smaller cell arrays. In order to monitor cell metabolic activity and differentiation in real-time, fluorescent reporter constructs were developed. Constituent promoter CMV-driven and differentiation-induced promoters fluorescence constructs were designed to visualize cellular metabolic activity of individual cells and to monitor MSC differentiation. The PPARγ, collagen 2a, and osteopontin promoters are indicative of adipose, cartilage, and bone differentiation.

Introduction

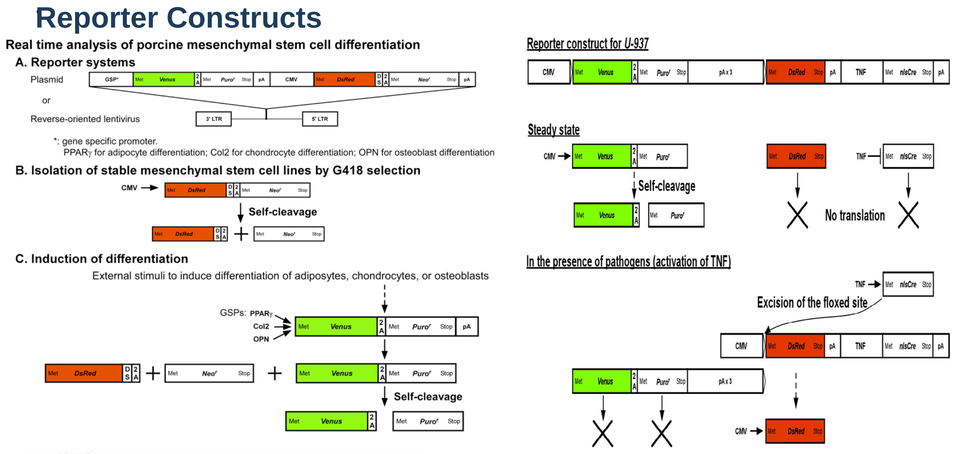
- Stem cell niche environments are complex 3D environments that maintain self renewal and direct differentiation of stem cells through a combination of signaling molecules, cell-cell interactions, and ECM interactions.
- The niche environment is poorly recapitulated in traditional 2D culture systems. Several cell types, including stem cells, display different phenotypes cultured in 3D vs. 2D.
- A limitation to 3D scaffolds is the lack of precise placement of cells and signals within the scaffold. Thus, we are using optical tweezer technology to place cells and signals within PEGDA and alginate 3D scaffolds.

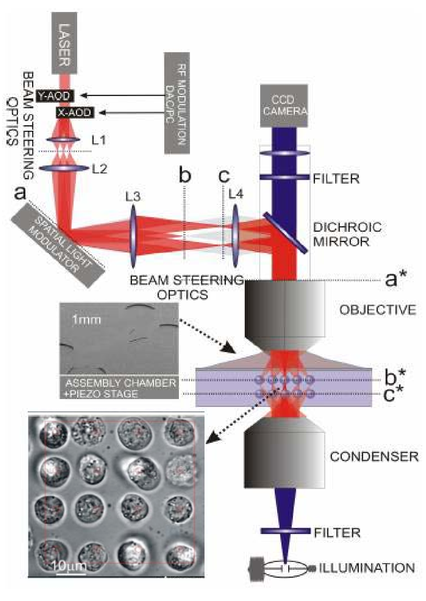
Objectives

- To create reporter systems to monitor stem cell self renewal and differentiation in real time.
- To recreate 3D infectious and stem cell niche environments using laser manipulation in conjunction with rapidly polymerizable scaffolds
- To optimize scaffolds for laser manipulation and cell activity.
- To observe cell signaling and differentiation in 3D hydrogel scaffolds

Materials and Methods

Reporter Constructs





Cells were transfected using the Amaxa Nucelofector Device and the corresponding Amaxa nucleofector kit for the cell line being transfected.

Optical Trapping

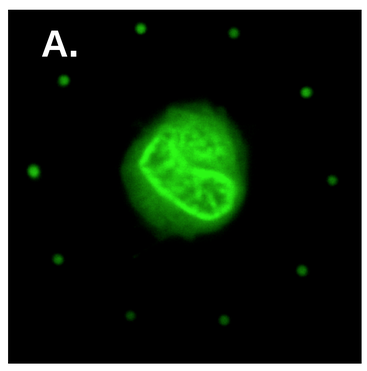
Cells were arrayed using a Zeiss neo-fllour 63x objective and a time averaged power of 6 mW per trap.

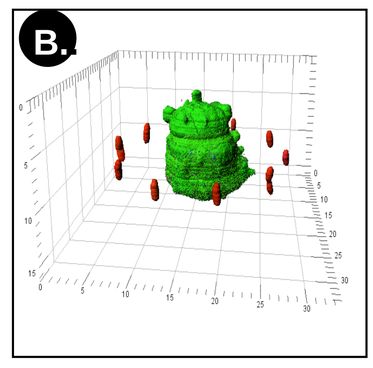
Cell Encapsulation

Cells were trapped in 5% 3.4k poly (ethylene glycol) diacrylate solution with 0.2% 2-hydroxy 2-methylpropiophenone as a photoinitiator. Hydrogels were crosslinked by exposing solution to a focused UV beam at 6mw/cm^2 for 5-20 seconds. For alginate encapsulation, cells were resuspended in a 2% alginate solution and polymerized with .5M $CaCl_2$ solution.

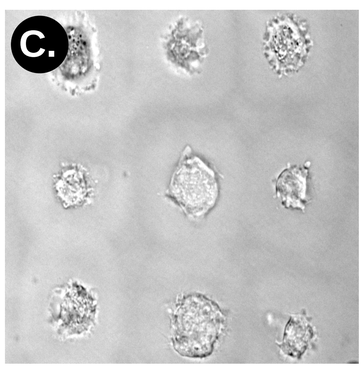
Results

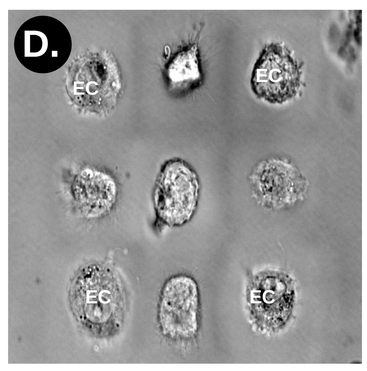
Heterotypic Array of U937 and ATCC 25922 pathogenic *e. coli* to study signaling in 3D environments. Panel A. Syto-9 stained U937 and *e. coli*. **Panel B**. 3D rendering of Panel A. U937 cells are rapidly stimulated to differentiate into the macrophage lineage by LPS secreted from 25922 E. coli.

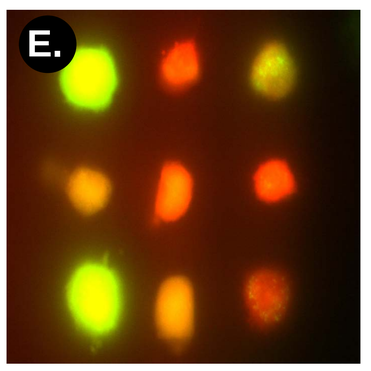




Homotypic and Heterotypic Arrays to Recreate the Stem Cell Niche. Panel C. Homotypic array of MSCs. **Panel D and E.** Heterotypic array of porcine MSCs and porcine aortic endothelial cells (EC). Prior to arraying, MSCs were stained with rhodamine (red fluorescence) and ECs stained with calcein AM (green fluorescence). To minimize cell exposure to lasers, cells were encapsulated individually in to the array.

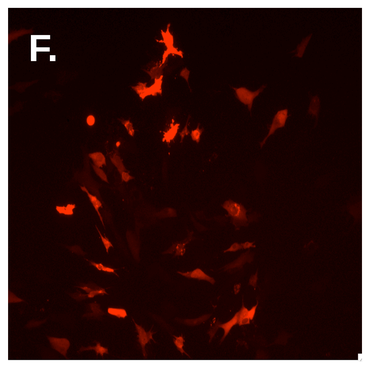


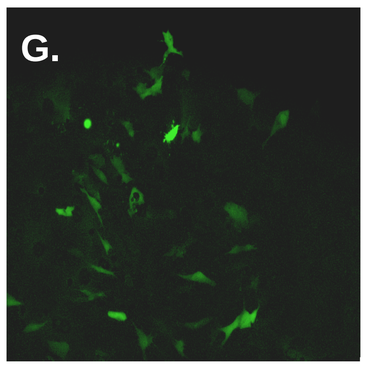




Reporter Constructs for Monitoring Differentiation in Real Time

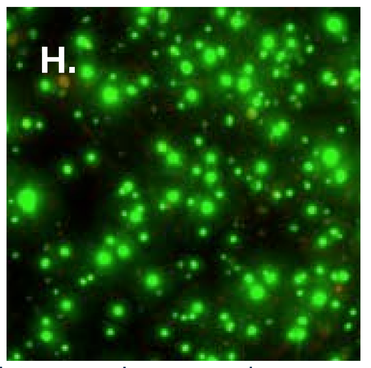
Panel F. Porcine mesenchymal stem cells nucleofected with a PPARγ reporter construct. Positively transfected cells show DsRed fluorescence. **Panel G**. Stem cells in A. differentiated for 6 days with adipogenic induction media. GFP fluorescence is driven by the PPARγ promoter that is positively up regulated in cells differentiating towards the adipogenic lineage.





Scaffold support of cellular activity

Panel H. Mesenchymal stem cells encapsulated within 2% alginate scaffold and differentiated with adipogenic media for 14 days. At the conclusion of the experiment, cells were stained with a live/dead staining kit. Live cells are stained green and dead cells are red. This demonstrates alginates capability to support cell activity.



Conclusions

- U937, ATCC25922, MSCs, and PAECs were successfully manipulated using laser tweezers into several arrays and encapsulated within PEGDA and alginate scaffolds.
- PPARγ reporter systems provide a real-time readout of adipogenic differentiation. OPN and collagen IIa constructs to monitor bone and cartilage differentiation are in construction.
- Cells can be successfully manipulated by laser tweezers in up to 5% PEGDA and 2% alginate prepolymer solutions. Alginate scaffolds support cell activity for at least 14 days.

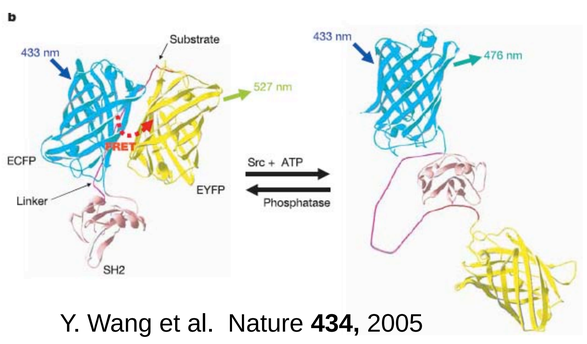
Ongoing Research

•SRC-FRET constructs to monitor cell signaling

SRC is a receptor tyrosine kinase that is activated in several signaling pathways, including those for LPS response and growth factor signaling. The conformational change during phosphorylation of SRC results in a change in FRET signal. This system provides a rapid readout of cellular signaling.

•Alternative Cell Lines to Monitor Signaling

Due to the difficulty of transfecting U937 cells, we are exploring several different cell lines that respond to LPS and phorbol esters. To date, we have successfully transfected SRC fret constructs into the easily transfected HEK TLR4/CD14/MD2 cell line and Kg-1 cells. The TLR4, CD14, and MD2 are required for LPS response in HEK cells.



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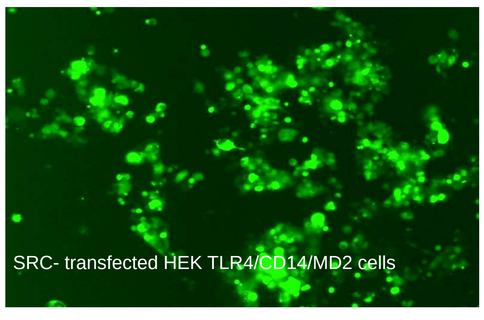

Acknowledgements

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