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Center for Biologics Evaluation and Research

ADVANCING REGULATORY SCIENCE

Supporting accurate interpretation of microfluidic system analysis of multipotent stromal cell morphology

A microfluidic system provides a confined environment for high-throughput analysis of multipotent stromal cell (MSC) morphology, which can reflect behavior. This system is widely used to create 3-dimensional tissue microenvironments that more closely mimic the environment of MSCs in the human body than do wells on plastic dishes. But FDA researchers found that the commonly used technique of sterilizing the microfluidic channels with UV light produces changes in the platform that can alter cell morphology. Understanding this issue will contribute to the accurate interpretation of microfluidics data used in the development of MSCs for regenerative medicine.

Adaptation of a Simple Microfluidic Platform for High-Dimensional Quantitative Morphological Analysis of Human Mesenchymal Stromal Cells on Polystyrene-Based Substrates

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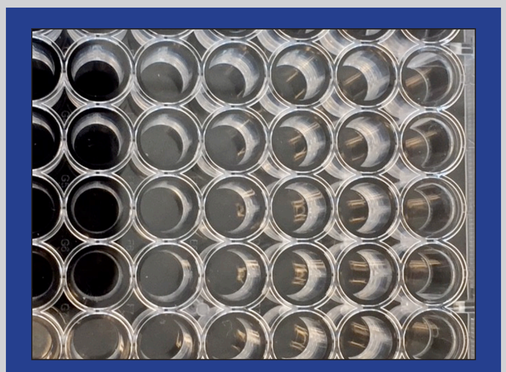
Heterogeneity of MSCs is a challenge to product development

- MSCs are being investigated in clinical trials to evaluate their ability to protect, restore, and repair tissues in the body.
- MSCs respond to growth factors by differentiating into cartilage, bone, or fat or by developing immunosuppressive functions.
- MSC subpopulations, as well as MSCs derived from different donors, display different capacities to proliferate and differentiate.
- Previous FDA studies showed links between changes in MSC morphology and the ability of the cells to develop certain abilities (e.g., laying down minerals that support bone growth, developing immunosuppressive activity).
- High single-cell resolution of morphological characteristics allows better understanding of the cellular heterogeneity of different MSC populations obtained from different donors and/or different manufacturing conditions.

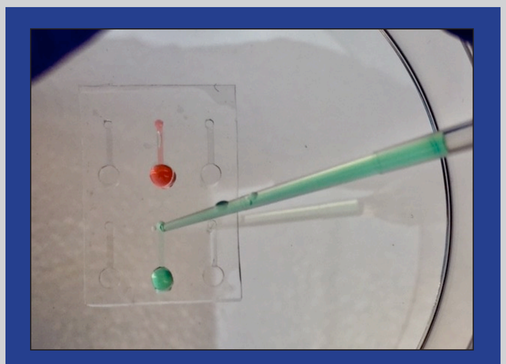
Limitations of small wells used for high-throughput MSC analysis

- Analysis of MSC differentiation capacity has often been done by placing cells in small wells on plastic plates to enable high-throughput analysis with powerful imaging devices.



The restrictive environment of wells can trigger MSC behaviors that are artifacts of the technique.

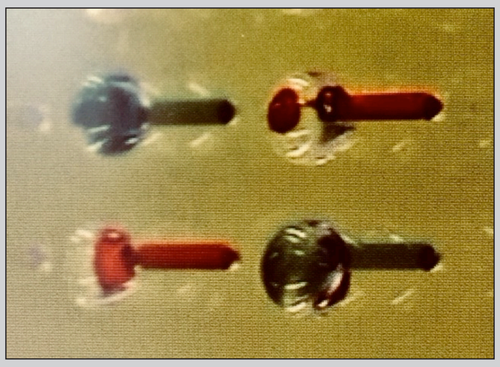
- The environment of small wells on plastic plates limits the ability of MSCs to simulate normal behavior in the human body.
  - Large volume of culture medium over MSCs placed in wells dilutes substances released by cells that are necessary for differentiation of cell populations.
  - Thermal convection continually displaces growth factors, reducing their ability to stimulate cells.



Demonstration of sample solutions being pipetted into channel opening (small opening at the left side of the channel) and moving under pressure to reservoir (large opening at the right side of a channel). The small bore of the channel provides an environment that is more confined than are open wells in plastic dishes.

Microfluidics: Scaling to the human environment

- Pipetting solutions of cells into a microfluidics system rather than into wells on plastic plates puts them into an environment on a physical scale more like that in the body (e.g., blood vessels).
- Fluid pipetted into opening of a microchannel is driven by pressure through the channel to a reservoir at the opposite end.
- Many microfluidics systems are constructed of silicon-based soft plastic (polydimethylsiloxane, PDMS) channels bonded to a polystyrene (PS) surface.
- Microchannel environment enhances sensitivity of assays compared to open-well environment.
  - May help researchers detect differences in cellular responses to surrounding tissues within channel.
  - May help detect different cell responses to certain manufacturing conditions.



UV sterilization of plastic-based microfluidics channels can compromise MSC studies

- FDA scientists exposed microfluidics PS platform substrates to sterilizing UV light before seeding stimulated MSCs into them to simulate preparation for high-throughput analysis of cellular behavior within the microchannels.
- UV light treatment of PS platforms caused changes in the shapes and sizes of MSCs that could confound behavioral analyses of these cells.
  - Increased hydrophilicity of PS changed MSC shape.
  - MSC morphological changes were greater when cells were at lower density in microfluidic straight channels than when seeded in higher density.
  - MSCs seeded in UV-treated microfluidics platforms displayed much higher sensitivity to changes in substrate properties than when in wells.
- Increasing cell density and serum concentration can reverse effects of UV sterilization cell interaction with tissue-culture-treated platform.

The use of microfluidic systems for high-throughput analysis has the potential to enable rapid, accurate identification of cellular characteristics of individual MSCs that predict differentiation into specific, functional cells. However, sterilization of these platforms using UV light produces changes in their polystyrene makeup that can produce behavioral artifacts in the cells. This FDA study demonstrates that with appropriate attention to these effects, microfluidic devices can be adapted as simple platforms for high-throughput analyses of MSC morphology and for studying cell-material interactions.