Stem cell proliferation and differentiation observed within hydrogel

April 11 2013

Stem cells can be coaxed to grow into new bone or new cartilage better and faster when given the right molecular cues and room inside a water-loving gel, researchers at Case Western Reserve University show.

By creating a three-dimensional checkerboard—one with alternating highly connected and less connected spaces within the hydrogel—the team found adjusting the size of the micropattern could affect stem cell behaviors, such as proliferation and differentiation.

Inducing how and where stem cells grow—and into the right kind of cell in three dimensions—has proven a challenge to creating useful stem cell therapies. This technique holds promise for studying how physical, chemical and other influences affect cell behavior in three-dimensions, and, ultimately, as a method to grow tissues for regenerative medicine applications.

"We think that control over local biomaterial properties may allow us to guide the formation of complex tissues," said Eben Alsberg, an associate professor of Biomedical Engineering at Case Western Reserve. "With this system, we can regulate cell proliferation and cell-specific differentiation into, for example, bone-like or cartilage-like cells."

Oju Jeon, PhD, a postdoctoral researcher in Biomedical Engineering, pursued this work with Alsberg. Their work is described April 11, 2013 in the online edition of *Advanced Functional Materials*.

Hydrogels are hydrophilic three-dimensional networks of water-soluble polymers bonded, or crosslinked, to one another. Crosslinks increase rigidity and alter the porous structure inside the gel.

Alsberg and Jeon used a hydrogel of oxidized methacrylated alginate and an 8-arm poly(ethylene glycol) amine. A chemical reaction between the alginate and the poly(ethylene glycol) creates crosslinks that provide structure within the gel.

They tweaked the mix so that a second set of crosslinks forms when exposed to light. They used checkerboard masks to create patterns of alternating singly and doubly crosslinked spaces.

The spaces, which varied in size at 25, 50, 100 and 200 micrometers across, were evenly singly and doubly crosslinked.

Human stem cells isolated from fat tissue were encapsulated in the singly and doubly crosslinked regions. The doubly-crosslinked spaces are comparatively cluttered with structures. The cells grew into clusters in the singly-crosslinked regions, but remained mostly isolated in the doubly crosslinked regions.

The larger the spaces in the checkerboard, the larger the clusters grew.

Cells were cultivated in media that promote differentiation into either bone or cartilage.

In both the singly and doubly crosslinked spaces, stem cells increasingly differentiated according to the media composition as the space size increased. The results were more dramatic in the singly-crosslinked spaces.

"Potentially, what's happening is the single-crosslinked regions allow

better nutrient transport and provide more space for cells to interact and, because it's less restrictive, there's space for new cells and matrix production," Alsberg said. "Cluster formation, in turn, may influence proliferation and differentiation. Differences in mechanical properties between regions likely also regulate the cell behaviors."

The researchers are continuing to use micropatterning to understand the influences of biomaterials on stem cell fate decisions. This approach may permit local control over cell behavior and, ultimately, allow the engineering of complex tissues comprised of multiple cell types using a single stem cell source.

Provided by Case Western Reserve University