Thermally responsive chitosan as an injectable matrix for therapeutic delivery of mesenchymal stem cells

Jiyoung M. Dang, Sing Yian Chew*, Kam W. Leong
Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205
*Department of Material Sciences and Engineering, Johns Hopkins University, Baltimore, MD 21218

Statement of Purpose: The immense impact of stem cell research is evident in regenerative medicine technologies. Adult stem cells, though less plastic than their embryonic counterparts, nevertheless have shown the capacity to provide therapeutic benefit to injured tissues *in vivo* (1). These MSCs are generally injected directly into the site of injury, however, we propose that delivery of these cells in a defined matrix can provide additional benefits. The matrix is a stable medium in which to retain cells at the site of delivery. Additionally, biologically active features can be incorporated into the matrix, such as specialized matrix proteins and growth factors, to further promote tissue regeneration.

We are investigating the therapeutic use of MSCs for the treatment of degenerative disc disease. We propose that delivered MSCs will be able to provide support to the native cells of the intervertebral disc (nucleus pulposus cells, NPC, and annulus fibrosus cells, AFC) via matrix production and paracellular growth factor signaling. Using a thermally responsive polymer, hydroxybutyl chitosan (HBC), a multifaceted approach can be taken with an injectable delivery of MSCs to the inner core of the disc (NP) and a cell sheet "patch" of MSCs to wrap and support the outer shell (AF).

Thermally responsive polymers are an ideal candidate class of polymers for injectable cell-based therapies. Gel formation of HBC can be triggered by body temperature, allowing for minimally invasive, non-toxic cell delivery (2). These polymers are also amenable to unique culture systems, such as cell sheets, providing flexibility in production of tissue-like structures *ex vivo* (3).

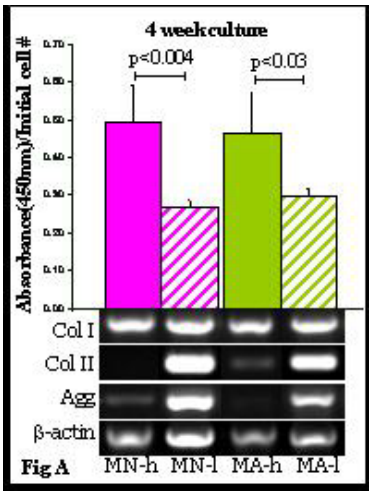

Fig A

This study focuses on the potential cell-cell and cell-matrix interactions that exist between MSCs, with a broader scope of these series of experiments being to highlight the versatility of the polymer to assist in development of a therapeutic treatment option for tissue regeneration.

Methods: Polymer purification and gel production was conducted as outlined in reference (2). HBC fibers were generated by electrospinning. WST-1 proliferation assay (Roche) was used to quantitate cell metabolic activity. RT-PCR gene expression analysis was performed on total cell RNA extracts.

Results / Discussion: To simulate the potential interactions between cells native to the intervertebral disc and MSCs delivered in HBC gels, two gel configurations were studied. One allows for direct cell contact between MSCs and AFCs or NPCs cultured in a single gel while another confines MSCs and AFCs or NPCs to separate gel layers, in order to determine if direct contact between the cell types is necessary to have a therapeutic effect.

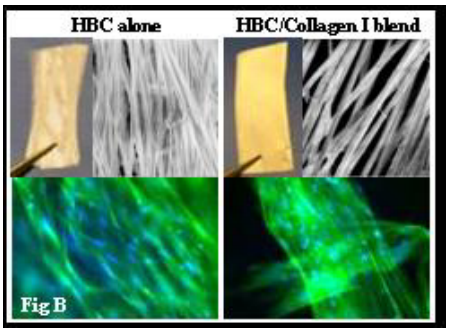

Fig B

MSC and NPC or AFC cultured within a single HBC gel (Fig A: MN-h and MA-h, respectively) displayed a significantly higher metabolic activity as compared to MSC and NPC or AFC confined to separate gel layers (Fig A: MN-l and MA-l, respectively). The same culture conditions but using a collagen blended HBC gel were also investigated. For cultures in collagen blended gels, no significant differences were seen in metabolic activity for all cell culture combinations, however, these cells were more proliferative than their HBC counterparts. These results indicate that direct contact between the two cell types is crucial and can result in elevated metabolic activity when cells are in the presence of a less-than-ideal matrix, such as that found in a degenerated disc.

When analyzing gene expression of key matrix proteins, however, MSC and NPC or AFC cultures in segregated gels show upregulated levels of matrix protein production as compared to their single gel counterparts. This trend is observed for both HBC and HBC/collagen blended gel cultures. Therefore, indirect influences from the MSCs. such as growth factor release, or direct contact between the cell types leads to overall decreased matrix production.

MSCs cultured on HBC or HBC/collagen blended nanofibers have the ability to align and elongate in the long axis direction of the nanofibrous surface. These cells produce normal collagen based matrices and display morphologies similar to the laminar AF matrix structure.

Conclusions: MSCs have the potential to promote nucleus repair with soluble factors promoting expression of specialized matrix proteins while direct contact increases metabolic activity. Further studies are ongoing to investigate this phenomenon using various dynamic matrix forms generated using the HBC polymer. These complex interactions between MSCs and disc cells must be taken into consideration when developing a therapeutic system for intervertebral disc regeneration.

References: (1)Pittenger MF, Martin BJ. *Circ Res* 95, 2004. (2)Dang JM, et. al. *Biomaterials* 27, 2006. (3)Yang J, et. al. *Biomaterials* 26, 2005.