Transfected Stem Cell Delivery via Thermoreversible Hydrogel for Improved Cardiac Function

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Statement of Purpose: The intent of this research is to improve cardiac function after an ischemic event through the application of transfected bone marrow derived mesenchymal stem cells in a thermo-reversible hydrogel scaffold.

While the use of cells suspended in saline is used, it is not perfect; only a limited number of cells may engraft at the implant site, with one study finding that only 10% of injected cells were retained 30 minutes after a myocardial injection (Hudson et al., 2007, J Surg Res, 142, 263-7). The obvious conclusion was that the "delivery strategy will need to be modified if more cells are to be retained within the target organ." The hydrogel scaffold will fit this purpose.

Utilizing cells transfected with a growth factor encoding plasmid may improve therapeutic efficacy. Growth factors serve to promote survival of native cells and induce angiogenesis. Multiple growth factors are investigated here for their effects on HUVEC cells and MSCs in vitro.

The MSCs are also examined to ensure that neither the transfection nor suspension in the scaffold affect the ability of the cells to differentiate, or induced differentiation.

Methods: PoligoGel is a novel high-molecular weight thermosensitive hydrogel developed by SamYang of Korea for the delivery of small molecules. It is based on multiple alternating blocks of poly(ethylene glycol) and poly(propylene oxide) or poly(butylene oxide). The LCST is about 32 Celsius. Here it is studied for the embedding of rat bone marrow derived mesenchymal stem cells (rMSCs).

MSCs were grown in Alpha-MEM (Invitrogen) with 1x Pen/Strep (Invitrogen) and 20% FBS (bioWest). An EGM-2 bullet kit from Lonza was used for growing the HUVECs.

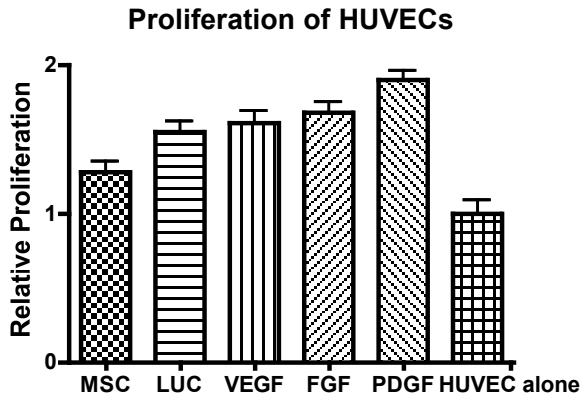
Assessing the viability of the cells was accomplished by either Trypan Blue (Sigma) staining, or alamarBlue (Invitrogen) following the manufacturers recommended protocol.

Growth factors VEGF, bFGF, and PDGF-CC were cloned from 1st strand cDNA from human placenta (Spring Bioscience) and inserted into the PCI plasmid (Promega Corp). Luciferase was cloned into the PCI plasmid as a control. Plasmid DNA was purified using Pure-Link Columns (Invitrogen).

Transfections were conducted using PEI, or other cationic polymers in serum free conditions.

RT-PCR was used to look at the expression of several different genes indicative of differentiation toward either adipogenic or osteogenic fates after exposure to PoligoGel and transfection. Cells placed in differentiation media and allowed to differentiate were used for controls.

Results: PoligoGel was first checked for toxicity and was found to be non-toxic at all concentrations tested, up to 10%. While not cytotoxic, at gelling concentrations, >5%, it did impair cell proliferation. This was true for both cells embedded in PoligoGel, as well as those under the gel on a tissue culture substrate. Trypan blue was used to verify viability in the absence of high proliferation. Embedded rMSCs did not suffer any significant toxic effects up to concentrations up to 1 million cells per ml, at which point a decrease in viability to 84% was observed based on Trypan blue staining. Transfected cells embedded in the hydrogel showed the ability to express the added protein, as verified by Luciferase expression. The release of growth factors from transfected stem cells was able to induce a greater growth response in HUVECs than non-transfected cells, or media alone as measured by alamarBlue. ANOVA indicated a significant difference, and Bonferronni posttests indicated PDGF-CC significantly enhanced proliferation above MSCs alone or no MSC treatment at a $p<0.001$.



RT-PCR results looking for differentiation did not show any effects due to being embedded in the gel. In each instance the results were similar to that of the controls consisting of cells plated at the same time as the experimental cells, but not exposed to PoligoGel. It was also possible to induce differentiation on cells removed from the gel to either adipogenic or osteogenic fates, indicating that the cells had not lost their multipotency.

Conclusions: PoligoGel has shown potential to be a safe and effective vehicle for the delivery of transfected rMSCs. It is non-toxic to all cells tested, and there are also no deleterious effects on the stem cells differentiative capacity. The use of transfected cells was also shown to influence the proliferation of HUVECs in vitro.

This treatment has the potential to improve cardiac function after implant by stimulating new vessel formation due to the secreted growth factors, and potential integration of the stem cells into the implant site.