Stem Cell treatment for Peripheral Arterial Disease - comparison of plain and encapsulated X-ray visible Mesenchymal Stem Cell transplants

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Introduction:

Stem cell transplantation is an experimental treatment for Peripheral Arterial Disease (PAD) that has the potential to restore a sufficient blood supply to the ischemic tissue by enhancing arteriogenesis. Two major problems with stem cell therapies are the poor cell survival and difficulties with visualization of cell delivery and engraftment. These issues have provided a motivation for the development of a microencapsulation method, which can protect cells from early destruction and simultaneously facilitate monitoring of cell distribution.

The presented work compares unencapsulated cellular therapy to microencapsulation therapy. Non-invasive imaging was used to monitor the cell delivery as well as to serially interrogate the degree of cellular retention and engraftment. In conventional direct superparamagnetic iron oxide labeling of bone marrow-derived stem cells, MRI was used to visualize the development of new collateral vessels as well as the effect on tissue perfusion downstream of ischemic tissue bed in a PAD animal model. In addition, more traditional X-ray angiography was used to assess arteriogenesis. Using microencapsulated stem cell therapy with an X-ray visible agent in the same PAD animal model, traditional fluoroscopic X-ray imaging techniques were used to assess arteriogenesis. Histopathology was used to validate both methods and determine whether microencapsulation was superior to "naked" stem cell administration.

Methods:

Mesenchymal stem cells were isolated from bone marrow of male New Zealand White rabbits and expanded for 3 passages. For magnetic labeling of plain cells, the magnetoelectroporation (MEP) was used at iron concentration of 2 mg/ml[1]. For microencapsulation, the classical alginate method[2] was used with the addition of barium sulfate to create X-ray visible capsules (XCaps)[3].

Hindlimb ischemia was induced by an endovascular method where thrombogenic platinum coils were deployed in the superficial femoral artery[4]. Twenty-four hours after ischemia induction, female rabbits were randomized to receive $13x10^6$ magnetoelectroporated (MEP)-labeled MSCs (n=6), XCaps with MSCs (n=5), XCaps without MSCs (n=6), or sham injections (0.3 mg of Fe/ml of PBS) (n=6) divided into 6 injection sites in the ischemic medial thigh.

MRI was performed on clinical 3T Philips Achieva scanner in rabbits that received MEP-labeled MSCs and sham injections immediately prior and after injection and at 1 and 2 weeks post-injection. An adiabatic T2-prepared, ECG-gated MRA was acquired to assess collateral vessel development with the following imaging parameters: TR/TE=14/3.8 ms; FOV=270x216 mm^2; acquired voxel size = 0.34x0.35x1.5 mm^3; TFE factor of 12; and FA=20°. To document ferumoxide injection sites, axial, fast gradient echo images were acquired with imaging parameters of: TR/TE=18/11 ms, spatial resolution=0.5x0.63x3 mm^3, 15° flip angle; and 18-20 slices. At two weeks post-injection, X-ray angiography was performed to confirm MRA findings. Subsequently, the animals were humanely euthanized, and tissue was harvested for histological analysis.

In the XCap groups, the injection sites were documented in AP and lateral fluoroscopic views (Infinix CC-I, Toshiba) immediately after injection and at 2 weeks after occlusion. X-ray angiography was also performed to investigate collateral vessel development. In all animals, X-ray angiograms were evaluated using a modified TIMI frame count, i.e. comparing the difference in time of filling of the popliteal bifurcation between the right non-ischemic and left ischemic limb.

Results:

MEP labeling resulted in efficient magnetic labeling with <5% decrease in cell viability. In vitro analysis of encapsulated MSCs showed cell viability was unchanged by the addition of 10% barium sulfate over 30 days (Fig 1).

High resolution MRI confirmed the presence of all 6 injection sites at 2 weeks in the rabbits receiving MEP-labeled cells, but the injection sites were not visible in the sham injected animals. The X-ray appearance of XCaps with and without MSCs was not difference immediately after injection and remained visible for 2 weeks post-injection. The proximity of the MEP-labeled MSCs to the neovasculature based on T2-prep MRA could be demonstrated as well as the XCap location relative to X-ray angiography. MRAs at 2 weeks demonstrated a high agreement with X-ray angiography (Fig.2). Furthermore, the incorporation of the contrast agent laden cells into the small vessel walls was confirmed with anti-dextran immunohistochemistry, whereas no anti-dextran staining could be demonstrated in the ferumoxide sham injected animals at 2 weeks. TIMI frame counts showed higher therapeutic effect in animals that got encapsulated MSC than naked MSCs (4.2 ±0.8 s vs. 7.7 ± 3, Fig.3).

Conclusion:

While naked MSCs can secrete cytokines as well as engraft and incorporate into the vessel walls, encapsulated MSCs are restricted to cytokine production solely. However, non-invasive imaging demonstrated a more robust angiogenic response with encapsulated MSCs relative to naked MSCs. Further investigation of putative cause of the enhanced therapeutic effect of microencapsulation will need to be performed to determine whether enhanced cell survival, cytokine production, or both.

References:

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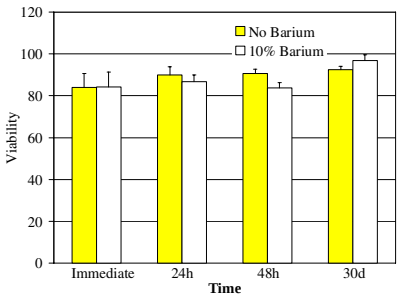

Fig.1. Comparison of the cell viability in pure alginate microcapsuls (yellow) and 10% barium X-ray visible capsules (white).

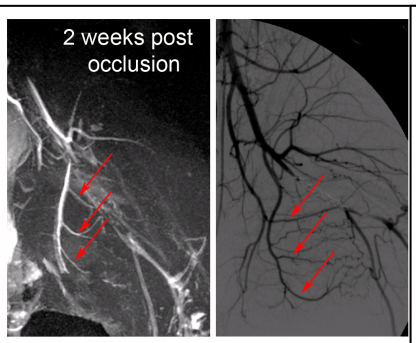

Fig.2. MRAs at 2 weeks demonstrated a high agreement with X-ray angiography

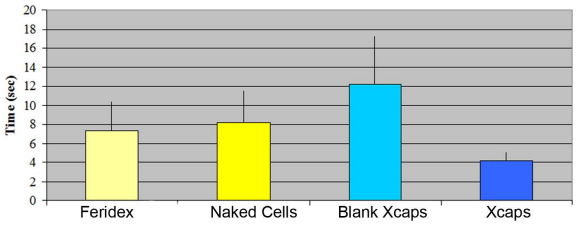

Fig.3. TIMI frame counts revealed higher therapeutic effect in animals that got encapsulated MSCs than naked MSCs.