

Fate Of Culture-Expanded Mesenchymal Stem Cells In The Microvasculature: In Vivo Observations of Cell Kinetics

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Background: Vascular delivery of mesenchymal stem cells (MSC) for therapeutic purposes is under clinical investigation. Little is known however about the microvascular fate of MSCs.

Methods: We used intravital microscopy of rat cremaster muscle microcirculation to track intra-arterially delivered MSCs. Rat MSCs expanded in adherent cultures (average diameter 23 μm) were bolused into the ipsilateral common iliac artery.

Results: Interrogation of an arteriole-venule pair revealed that $92\pm 7\%$ ($n=6$) of MSCs arrest and interrupt flow during first pass at the precapillary level, resulting in decreased flow in the feeding arteriole (velocity decreased from 6.3 ± 1.0 mm/s to 4.6 ± 1.3 mm/s, $p<0.001$). When intravital microscopy was performed 3 days following injection, the number of MSCs in the cremaster further decreased to 14% of the initial number, due to cell death *in situ*. *In vivo* labeling of the basement membrane revealed that at 1 day, the surviving cells were spread out on the luminal side of the microvessel, while at 3 days the few surviving cells integrated in the microvascular wall with restoration of flow. MSC deformability evaluated using filtration through polycarbonate membranes revealed that the cortical tension of MSCs (0.49 ± 0.07 dynes/cm, $n=9$) was not different from that of circulating mononuclear cells (0.50 ± 0.05 dynes/cm, $n=7$).

Conclusions: Despite their deformability, intra-arterially delivered MSCs entrap at the pre-capillary level due to their large size, with a small proportion of surviving MSCs integrating in a perivascular niche. We are currently exploring expansion of bone marrow cells in suspension cultures using microcarrier beads, which allows for preservation of the *in vivo* MSCs phenotype (diameter 11-12 μm).