SUPPLEMENT MATERIAL
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Intravital Microscopy

Acute experimental protocol

Fluorescently labeled MSCs were re-suspended in 1 ml PBS and slowly injected in the iliac artery over 30 seconds. An inverted microscope (Nikon Eclipse or Olympus IX81) coupled to a CCD camera (Orca, Hammamatsu) was used for direct visualization of the cremaster microcirculation. A microvascular pair consisting of an arteriole and its venule was observed continuously during cell injection. Fractional plugging (F_p) was calculated as the difference between the number of MSCs entering and exiting the arteriole and venule, respectively, divided by the number entering the arteriole. Velocity in the artery was recorded prior to and 15-25 minutes following cell injection using an RBC velocimeter (CircuSoft, Hockesin). Fluorescent cells identified in the area of interrogation were observed for up to 90 minutes. In separate experiments (n=4 rats), prior to the cell injection the cremaster muscle was pre-treated with topical 10 μM sodium nitroprusside (SNP) and an intraarterial bolus of 200 μl to study the effect of maximal vasodilatation on the Fp. The vasodilatory effect of the SNP was confirmed by measuring the diameter of a resistance arteriole before and after the drug administration.

Chronic experimental protocol

For longer-term examination of cell fate, separate experiments were performed in which the animals were allowed to recover after intra-arterial MSC infusion. Lewis rats were used to avoid an immunological rejection response. At 6, 24, 48 or 72 hours after cell

injection, the rats were prepared for intravital microscopy as described above. An upright BX51 Olympus microscope coupled to a digital camera (DP71, Olympus) was used for multicolor fluorescence imaging. The cells in 15 random low-magnification fields per animal (3.6 mm^2/field, with a total exposed cremaster area of 2-3 cm^2) were counted, then the average count per field was normalized to the number of cells injected (in millions). To anatomically localize the position of MSCs relative to the vessel wall, in a subset of rats (n=3 each at 24 and 72 hrs), microvascular basement membrane was stained with 30 μg/ml rhodamine–labeled Bandeiraea Simplicifolia Lectin I (Vector Labs).

To rule out that the possibility that cellular fluorescence observed 3 days after MSC injection did not emanate from cells taking up CMFDA released by dying MSCs, non-viable MSCs were arterially injected in a separate set of rats (n=3). Just prior to injection, death of CMFDA-labeled MSCs was triggered *in vitro* by exposure to 10 mM H_2O_2. Using this oxidative stress, 85% of the MSCs were non-viable by the Trypan blue exclusion test while retaining their fluorescent label for at least 24 hours. To further assess the viability of the entrapped MSCs, double-labeling experiments were performed in 4 additional rats, whereby in addition to the CMFDA labeling, the cells were labeled with the nuclear stain DAPI (Invitrogen) at 50 μg/ml for 60 min. The presence of chromatin condensation on nuclear staining was interpreted to indicate non-viability of the MSC.

Cell deformability

Polycarbonate filters (Nuclepore®, Whatman), having uniform pores of known density ($10^5/cm^2$), were mounted downstream from a syringe pump (Harvard Apparatus). Pore size was chosen to be lower than the minimal cell size (5 μm for MNC, 10 μm for MSCs). The pressure proximal to the filter was monitored with a fluid-filled pressure transducer (PM01, WPI). A volume of 50-200 μl of PBS with cells at 1:1 ratio with the number of pores in the filter was introduced proximal to the filter and advanced under slow flow (120 to 230 μl/min x 3 minutes) to the filter, so as to completely plug the pores. The flow was increased every 1 minute up to 10 ml/min, leading to a step-wise increase in the pressure across the plugged filter; this in turns led to a progressively higher number of cells passing through the filter, with the number of cells traversing the filter at a given pressure depending on cell size and deformability. Because the resistance of the filter to vehicle (PBS) is known, the number of pores plugged at each step can be calculated. This fractional plugging was plotted against the pressure rise and the mean p_{yield} calculated (the pressure required to advance 50% of the cells through the filter). This relationship is logarithmic[1], with a number of pores remaining permanently plugged even at higher pressures. The mean cell size in the effluent was measured, and the deformability of the cells, expressed as cortical tension (τ_0), was determined using a formula derived from the cortical shell model[2], namely $\tau_0 = p_{yield}/ (2 \times (1/R_{pore} - 1/R_{cell}))$, where R_{pore} and R_{cell} represent the mean radius of the pores and the cells, respectively. Cell diameter was determined by obtaining phase-contrast digital images of the freshly trypsinized cells and using Image J software (NIH) to analyze particle size. The deformability of MSCs was compared to that of MSCs treated for 30 min with 20 μM

cytochalasin B (to induce actin cytoskeleton disruption) or 10 mM H_2O_2 (to induce apoptosis).

References

1. Eppihimer MJ, Lipowsky HH. The mean filtration pressure of leukocyte suspensions and its relation to the passage of leukocytes through nuclepore filters and capillary networks. *Microcirculation*. 1994; 4:237-50.
2. Evans E, Yeung, A. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. *Biophys J*. 1989, 56: 151-160.

Supplemental Video: Intravital DIC imaging of the cremaster muscle demonstrating lack of flow in a precapillary channel containing arrested MSCs. The video corresponds to Figure 1c.