Diabetic Wound Healing Response to CD-34-Sorted and Unsorted Adipose-derived Stromal Cells Delivered as Self Organizing Mesenchoid Bodies

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Introduction: Adipose-derived stromal cells (ASCs) appear to confer regenerative benefits when introduced into various injured tissues, including bone, cartilage, and myocardium. In addition to their documented developmental potential, ASCs have also been shown to secrete various angiogenic, mitogenic and anti-apoptotic growth factors. We therefore hypothesize that ASCs possess the ability to enhance healing of diabetic wounds. We previously developed a novel method for culturing ASCs as self-organizing mesenchoid bodies (SOMBs), and here we test their ability to expedite healing of full thickness dorsal skin wounds in diabetic animals, focusing on CD-34 as a putative marker of stem cell enrichment and wound healing efficacy.

Methods: Human ASCs were isolated from an elective lipectomy specimen using well-documented methods. Cells were cultured on plastic and sorted at P=3 for expression of CD-34. CD-34-positive, CD-34-negative, and unsorted ASCs were then grown as SOMBs (25,000 ASCs/SOMB) in suspension culture for 8 days in serum free medium. On Day 0, a single 1 cm diameter full thickness excisional cutaneous wound was made on the back of homozygous diabetic null mice. Each wound was randomly treated in a blinded fashion on post-wounding day 1 with 5 SOMBs delivered topically in ~20 μl PBS under a Tegaderm dressing. The resulting ASC treatment groups consisted of: CD-34-positive ASC SOMBs (N=7), CD-34-negative ASC SOMBs (N=9), and unsorted ASC SOMBs (N=4). Digital images were taken of each wound every 2 or 3 days until Day 21 and open wound area, expressed as a percentage of initial wound area, was quantified using ImageJ analysis software.

Results: Wound areas in all three experimental groups were statistically similar to one another at each timepoint, suggesting that prospective sorting based on CD-34 expression had no impact on the ASCs' ability to influence wound healing. In addition, healing rates in ASC SOMB-treated wounds were statistically similar to those in diabetic mice treated with vehicle control for the first week after wounding. However, by day 9, all diabetic wounds treated with ASC SOMBs were significantly smaller than those in diabetic mice receiving vehicle control and statistically similar to wild type *non-diabetic* mice (from a historical dataset).

Conclusion: We have shown that the administration of ASCs as SOMBs accelerates the wound healing process in diabetic mice compared to those receiving no ASC treatment. However, prospective ASC enrichment on the basis of CD-34 expression did not enhance this therapeutic effect. Although additional

studies are necessary, our findings suggest promise for ASCs as a novel therapeutic approach to healing difficult wounds.