Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds.

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Abstract:

The nonhematopoietic component of bone marrow includes multipotent mesenchymal stem cells (MSC) capable of differentiating into fat, bone, muscle, cartilage, and endothelium. In this report, we describe the cell culture and characterization, delivery system, and successful use of topically applied autologous MSC to accelerate the healing of human and experimental murine wounds. A single bone marrow aspirate of 35-50 mL was obtained from patients with acute wounds (n = 5) from skin cancer surgery and from patients with chronic, long-standing, nonhealing lower extremity wounds (n = 8). Cells were grown in vitro under conditions favoring the propagation of MSC, and flow cytometry and immunostaining showed a profile (CD29+, CD44+, CD105+, CD166+, CD34-, CD45-) highly consistent with published reports of human MSC. Functional induction studies confirmed that the MSC could differentiate into bone, cartilage, and adipose tissue. The cultured autologous MSC were applied up to four times to the wounds using a fibrin polymer spray system with a double-barreled syringe. Both fibrinogen (containing the MSC) and thrombin were diluted to optimally deliver a polymerized gel that immediately adhered to the wound, without run-off, and yet allowing the MSC to remain viable and migrate from the gel. Sequential adjacent sections from biopsy specimens of the wound bed after MSC application showed elongated spindle cells, similar to their in vitro counterparts, which immunostained for MSC markers. Generation of new elastic fibers was evident by both special stains and antibodies to human elastin. The application of cultured cells was safe, without treatment-related adverse events. A strong direct correlation was found between

the number of cells applied (greater than 1 x 10(6) cells per cm2 of wound area) and the subsequent decrease in chronic wound size ($p = 0.0058$). Topical application of autologous MSC also stimulated closure of full-thickness wounds in diabetic mice (db/db). Tracking of green fluorescent protein (GFP)+ MSC in mouse wounds showed GFP+ blood vessels, suggesting that the applied cells may persist as well as act to stimulate the wound repair process. These findings indicate that autologous bone marrow-derived MSC can be safely and effectively delivered to wounds using a fibrin spray system.