THE SUPERFICIAL ZONE CELLS INITIATE A WOUND HEALING PROCESS IN CANINE MENISCUS IN VITRO
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Introduction: The meniscus is a fibrocartilage that, unlike articular cartilage, can repair wounds. The meniscus is populated by distinct cell populations that include round or oval shaped fibrochondrocytes [1], fibroblast-like cells with several extended cytoplasmic processes [2], and superficial zone cells that are active in repair processes [3]. We set out to determine whether the initial cells that migrate into the wound site in the meniscus were endogenous to the tissue and, if so, from which population they derived. We explored this issue in an in vitro organ culture model of the meniscus in serum-free medium.

Methods and Materials: Whole knee joints were obtained from skeletally mature dogs. The medial menisci were removed under sterile conditions. Full thickness circular defects, 2 mm in diameter, were made in the mid substance of each meniscus using a sterile dermal biopsy punch. The plugs were rendered acellular by freezing and thawing five times in liquid nitrogen. The plugs were then reinserted into their respective holes in the meniscus. Menisci without surgical defects constituted controls. In a variant of this model, the meniscus was cultured without the vascular zone.

The meniscus were then cultured in serum free medium for 1, 2, 3, 4, 5, and 7 weeks. One meniscus from each group was removed and embedded in OCT, and frozen sections were made (5 μm) in both dorsal and coronal planes. Indirect immunohistochemistry was performed using monoclonal anti-alpha smooth muscle actin (αSMA), proliferating cell nuclear antigen (PCNA), and a polyclonal anti-Von Willebrand factor and an FITC conjugated anti-mouse and anti-rabbit (fab') secondary antibody. The primary antibody was omitted in negative controls. The cell nuclei were visualized using Vectashield with DAPI.

Results: Cell viability assays with MTT demonstrated that the cells in the menisci remained viable for up to 7 weeks in culture. The freeze-thawed meniscal plugs were completely devoid of cells at reimplantation into the holes in the tissue. An acellular circular zone in the meniscal tissue adjacent to the plug was evident in the first 1-2 weeks after wounding (Fig. 1). TUNEL/Annexin V assay demonstrated that the loss of cells in this region was by apoptosis. Cells were present in the crevice between the plug and the adjacent meniscus at 2 weeks (Fig. 1). The DAPI staining pattern suggested that these cells migrated into the crevice from the superficial zone and not from the body of the adjoining meniscus, as the zone in the meniscus adjoining the crevice was devoid of cells (Fig. 1). Cells had densely populated the plug by 3-5 weeks in culture (Fig. 2). By 6 weeks the plug looked like normal meniscal tissue, thickly populated with cells. Identical cell migrations into the crevice and plug were evident in menisci from which the vascular portion was removed.

The cells in the superficial zone and the cells in the crevice were positive for PCNA, confirming cellular proliferation (data not shown).

Discussion: Our previous study showed that superficial zone cells express smooth muscle actin and are active in wound repair in vivo [3]. Our data here show that:

(a) the migration of cells into the crevice of a wound, the first cellular event in wound healing, can take place in the meniscus in vitro;
(b) the initial cells to migrate in from the superficial zone, particularly on the outer femoral surface;
(c) vascularity does not appear to play a role in the initial phase of wound healing in the meniscus.

The superficial zone cells appear to have distinctive abilities for migration into the crevice and initiation of the very earliest phase of a repair response.

References:

Acknowledgments: This work was supported by NIH grants R01 AG 14342 and R21 AR 46991 to CAM.

49th Annual Meeting of the Orthopaedic Research Society
Poster #0716