

Characterization and Utilization of Mesenchymal Progenitor Cells Recovered with the Reamer-Irrigator-Aspirator

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INTRODUCTION: Trauma surgeons frequently need to repair large and difficult osseous defects. Central to this endeavour is a source of bone or bone precursors. The Reamer-Irrigator-Aspirator (RIA) is a new device that might supply these in a convenient and relatively non-invasive fashion. The RIA is inserted into the intermedullary canal and the “reamings” are harvested from an irrigating solution used in conjunction with the device. The reamings comprise osseous particles that are captured with a coarse filter, and “flow-through” that passes through the filter. While the osseous particles have been used to augment fracture repair, the “flow-through” has not been well characterized. Here we describe the isolation and characterization of mesenchymal progenitor cells recovered from reamings, and suggest ways in which they might be used to develop novel, intra-operative technologies for the efficient restoration of bone and other musculoskeletal tissues.

METHODS: Subjects (n=7) were 71-97 years of age, undergoing hip hemiarthroplasty. Osseous particles were collected by filtration and cells recovered by centrifugation, with or without Ficoll-gradient separation. Supernatants were assayed for BMP-2, IGF-1, FGF-2 and TGF- β_1 by ELISA. With certain reamings, osseous particles were placed into culture to allow the outgrowth of cells which were then harvested by trypsinising, and sub-cultured. The surface phenotype of the cells was analysed by FACS. Chondrogenesis, adipogenesis and osteogenesis were assessed using standard assays.

RESULTS: BMP-2 could not be detected in the reamings, but IGF-1 (4.9 ± 3.7 ng/ml), FGF-2 (430 ± 160 pg/ml) and TGF- β_1 (9.8 ± 5.9 ng/ml) were present. However, because of the diluting effect of the irrigant, these concentrations are relative. Filtrate cells were CD44+, CD90+, CD105+ and CD106+, but CD34- and CD45-. These cells were able to differentiate along adipogenic, chondrogenic and osteogenic lineages. Cells emerging from the osseous particles were indistinguishable from those recovered from the flow-through in terms of their morphology and ability to undergo multi-lineage differentiation. [1] The rich harvest of osteogenic cells was

remarkable, given the age of these patients. The impressive osteogenic potential of the reamings encourages us to develop methods that will allow the trauma surgeon using the RIA to isolate osteoprogenitors, commit them irreversibly to osteogenesis, and re-implant them within a single operation. Thus we are defining conditions that, within 2 hours, will commit mesenchymal progenitor cells irreversibly to osteogenesis. These include transfer of osteogenic genes, exposure to proteasome inhibitors and histone de-acetylase inhibitors, among others. As noted first by Leboy's group [2], human mesenchymal progenitors do not undergo osteogenesis in response to rhBMP-2, but they respond well to dexamethasone, BMP-6 and BMP-9. Preliminary data suggest that cells recovered from the reamings are readily transduced in suspension by adenovirus vectors.

DISCUSSION & CONCLUSIONS: The RIA provides the trauma surgeon with new possibilities for greatly enhancing bone healing in a convenient and affordable manner. The challenge is to define powerful osteogenic stimuli that will be effective within the short, intra-operative period.

REFERENCES: ¹ R. M. Porter, et al (2008) *J Orthop Res* Submitted.. ² D. L. Defenderfer, et al (2003) *J Bone Jt Surg* 85A: 19-28.

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