Biomaterial-Mediated Delivery of Uncultured Rat Bone Marrow Mononuclear Cells and Culture-Expanded Mesenchymal Stem Cells for Large Bone Defect Healing

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Introduction: Repair of critical-sized bone defects is a major clinical challenge. Stem cell-based biomaterial therapies have shown promise in enhancing bone healing. Marrow-derived, multipotent mesenchymal stem cells (MSC) are typically expanded vitro to obtain large quantities, but the process is lengthy and cost prohibitive. Potential advantages of using fresh, uncultured bone marrow progenitor cells include the heterotypic cell and paracrine interactions between MSC and other marrow-derived cells (including HSC/HPC and EPC). In this study, we tested the overall hypothesis that collagen-chitosan microbead encapsulation of a mixed cell population from fresh uncultured bone marrow mononuclear cells (BMMC) will display equivalent or enhanced osteogenic potential, relative to culture-expanded MSC-encapsulating microbeads. We further tested whether hypoxia further enhances initial cell survival and subsequent osteogenic differentiation.

Methods: Freshly isolated BMMC (25×10^6 cells/ml, Sprague-Dawley rats, 3-6 wks) or culture-expanded rat bone marrow-derived MSC (5×10^5 cells/ml, passage 4) were added to a collagen-chitosan (65/35 wt%) hydrogel mixture and fabricated into 3D microbeads by emulsification in PDMS and thermal gelation. Microbeads were cultured in control MSC growth medium in either 20% O_2 (normoxia) or 5% O_2 (hypoxia) for an initial 3 days. Subsequently, samples were cultured in control medium or osteogenic medium supplemented with ascorbate, β-glycerolphosphate, and dexamethasone. At day 1 and 21, microbeads were assessed for viability, total DNA and calcium content. Data are reported as mean ± SD and analyzed by Student's t-test (α=0.05). For histology, microbeads were sectioned and stained with H&E, Alizarin Red, and Von Kossa.

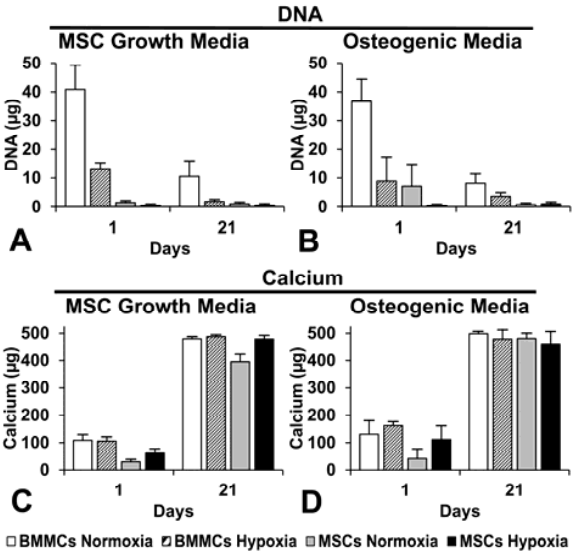

Fig. 1. DNA and calcium content in microbeads (n=4)

Results: At day 1, BMMC-microbeads cultured in hypoxia showed significantly reduced DNA content, compared to normoxia (**Fig. 1A-B**). BMMC-microbeads cultured for 21 days in either medium exhibited a marked reduction in DNA, relative to day 1 (**Fig. 1A-B**). Microbeads cultured for 21 days in either control or osteogenic medium, in normoxia or hypoxia, displayed statistically significant increases in calcium, compared to day 1 (**Fig. 1C-D**). Calcium levels in osteogenic medium were not different from control medium at day 21.

BMMC-microbeads cultured in hypoxia at day 1 contained notably more live than dead cells, compared to normoxia (**Fig. 2A**). At day 21, all BMMC-microbeads exhibited considerably more live cells (> 60%) than dead cells, and cells had a spread morphology in osteogenic culture. At day 21, all culture-expanded MSC-microbeads contained at least 72% live spread cells.

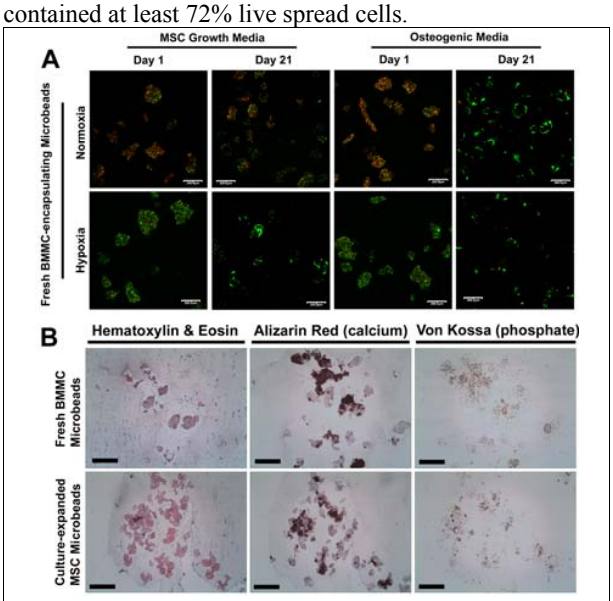

Fig. 2. A) Cell viability in BMMC-microbeads at day 1 and 21. B) Histology of hypoxia microbeads cultured in osteogenic media at day 21. Scale bar = 200 μm.

BMMC- and MSC-microbeads cultured in osteogenic medium for 21 days, either in hypoxia or normoxia, stained positive for calcium and phosphate (**Fig. 2B**).

Conclusions: Hypoxia enhanced initial progenitor cell survival of fresh BMMC in microbeads, but did not enhance osteogenic potential. As assessed by calcium content and histology, fresh uncultured BMMC show a similar degree of osteogenesis as culture-expanded MSC when cultured in collagen-chitosan microbeads. This is a profound effect since the heterogeneous BMMC group is estimated to contain only $1/10^{th}$ the number of MSC seeded into cultured MSC-microbeads. This study suggests that direct seeding of 3D collagen-chitosan microbeads with fresh BMMC holds promise as a potential therapy for bone defect repair.

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