Hypoxic Preconditioning of Mesenchymal Stem Cell Spheroids Stimulates Segmental Bone Defect Repair

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Purpose: The transplantation of mesenchymal stem cells (MSCs) holds great potential for use in musculoskeletal repair and addressing many shortcomings of existing therapeutic approaches. However, this approach is limited clinically due to poor cell survival and engraftment in vivo. Short-term preconditioning of MSCs under hypoxic conditions can promote cell viability and sustain persistence in vivo, and we demonstrated that MSCs formed into spheroids can be effectively deployed within a clinically relevant hydrogel to enhance their therapeutic potential for bone tissue engineering applications.

Method: Human MSCs were preconditioned in 1% O2 in monolayer culture for 3 days (PC3) or kept in ambient air (PC0), formed into spheroids of 3 different sizes (3,000, 10,000, or 15,000 cells/spheroid), and then entrapped at equal cell densities in alginate modified with the adhesive peptide RGD. We measured MSC viability and secretion of vascular endothelial growth factor (VEGF) over 4 days in serum-deprived/hypoxic conditions. Osteogenic potential of spheroids in alginate gels was determined by measuring alkaline phosphatase (ALP) activity and calcium deposition over 14 days in osteoinductive conditions. Alginate gels containing PC3 spheroids suspended at 30 million cells/mL were then implanted into a 6-mm critical-sized segmental defect in the right femora of athymic rats. Bone healing was evaluated over 12 weeks.

Results: The preconditioning of MSCs prior to spheroid formation exhibited beneficial effects on MSC survival and trophic factor secretion in vitro. Caspase 3/7 activity, an indicator of apoptosis, significantly decreased in PC3 groups compared to unconditioned controls. VEGF secretion, a key hallmark of proangiogenic potential, was greatest in PC3 spheroids. Secreted VEGF levels remained constant over 4 days for each spheroid size. MSCs formed into spheroids possessed robust osteogenic potential, with the largest spheroids (15,000 cells/spheroid) exhibiting increased ALP activity and calcium deposition over 14 days compared to other groups.

Conclusion: These data demonstrate that preconditioning of MSCs prior to spheroid formation and entrapment in RGD-modified alginate hydrogels promotes cell viability, proangiogenic potential, and bone healing. Our study is the first to demonstrate significant spheroid-mediated bone healing of a critical-sized segmental defect without the use of BMP-2.

See pages 401 - 442 for financial disclosure information.