ADULT STEM CELLS AND BONE: THE WHAT, WHERE, AND WHEN
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Key Points
- Definitions, Nomenclature, and Isolation
- Osteogenesis versus Ectopic Ossification
- Scaffold Carriers and Bioreactors
- The Future of Stem Cell Osteogenesis

There is a significant need for therapies to enhance fracture healing, especially in larger species like the horse. Traditional bone grafting techniques have limitations that may be avoided with stem cell tissue engineering strategies. While many of the early discoveries that serve as the foundation for the “stem” cells of today occurred within the last 70 years, sentinel findings by cell biologists and hematologists occurred in the 19th century. Hence, a long and convoluted history paired with an explosion of discovery over the last 20 years contributes to a plethora of definitions and standards within the stem cell field. For purposes of this review, the term multipotent stromal cells (MSCs) will be used to refer to cells that can be isolated by adherence to plastic and that possess the characteristics of self-renewal and the ability to differentiate into multiple tissue types including adipose, bone, and cartilage. Osteogenesis of two of the most common cell types harvested from adult mesenchyme, defined as originating from the embryonic mesoderm, will be covered. Hence, the key points will be specific to adipose- (ASCs) and bone marrow- (BMSCs) derived multipotent cells to address the need for therapies to enhance fracture healing.

An important consideration within MSC mediated bone formation is the difference between orthotopic and ectopic osteogenesis. Ectopic osteogenesis refers to ossification of tissue implanted outside of a normal site of osteogenesis (or outside of the implanted tissue origin). Orthotopic osteogenesis refers to bone formation in its correct anatomical location. Both orthopic and ectopic ossification models are used for many studies surrounding MSC osteogenesis. Implantation sites for experimental ectopic osteogenesis include subcutaneous, intramuscular, and less commonly, beneath the renal capsule. Ectopic sites allow a somewhat controlled environment for in vivo experimental bone formation with a relative lack of bone cytokine stimulation, cell-to-cell interaction with endogenous bone-forming cells, endogenous MSCs, and bone-stimulating mechanotransduction. However, these variables all contribute to bone regeneration in a normal fracture environment. Hence, while ectopic bone formation models contribute to optimization of MSC bone formation, the distinct biochemical and mechanical environment of orthotopic bone formation is perhaps most relevant to validation and implementation of MSC therapies.

Translation of MSC technology to augment patient fracture healing requires biocompatible scaffolds to carry and then support the cells following implantation. As such, there is significant effort directed toward developing scaffolds that promote MSC osteogenesis based on principles largely derived from autograft bone function and remodeling. Scaffolds can be divided into three major classes, polymers, ceramics, and metals, all of which have distinct advantages and disadvantages. The ideal scenario is for MSC-loaded implants to initiate and support bone regeneration in parallel with complete resorption of the scaffold. Scaffold carriers should encourage MSC adhesion, proliferation, and osteogenic differentiation. Implanted
scaffold should also support vascularization for an optimum osteogenic environment. Scaffold porosity, pore size, distribution and continuity dictate the interaction of scaffolds and transplanted cells with the environment. Pores less than 15-50 μm in diameter result in fibrovascular ingrowth; pores 50-150 μm encourage osteoid formation; and pores greater than 150 μm support mineralized bone ingrowth. Scaffolds are often coated with components like minerals, structural proteins, cytokines, hormones, and growth factors, to enhance implant integration and osteogenic commitment of native and implanted precursor cells. Scaffold conduction of mechanical stresses dictates, in part, formation of bone architecture. This, in turn, depends on scaffold composition and structure. Current research supports distinct differences between MSC cell types, and it is well established that patient age, gender, and health status all contribute to variability in bone forming capacity. There is no single scaffold-MSC combination that is likely to address every clinical scenario that might be addressed with this novel technology. Hence, MSC-scaffold customization will likely be necessary for optimal clinical application.

Bioreactors provide a mechanism to maintain cell-scaffold constructs in a biocompatible environment. The term “bioreactor” can refer to a wide variety of culture systems, both “static” and “dynamic”. Static culture systems are as simple as immersion of cell-scaffold constructs in medium on a culture plate within an incubator. Nutrient and waste removal is limited to diffusion, and three-dimensional constructs are often affected by lower nutrient and gas exchange in the center compared to the perimeter. In normal bone, no cell is more than 300 μm from a blood vessel, so static systems are not good representations of the natural environment. Hence, dynamic bioreactors are often used for MSC osteogenesis. They can be divided into three major categories, spinner flask, rotating wall, and perfusion. In a spinner flask, a stirring mechanism is used to mix the medium around the scaffolds. Nutrient and gas transport within the scaffold is due to diffusion, but fluid motion around the scaffold removes the concentration gradients between the inner and outer surfaces of the scaffold. The rotating wall bioreactor is a cylinder mounted horizontally with a core containing an oxygenator along the central axis of rotation. The space between the core and the outer vessel wall is filled with medium and the vessel is rotated. The angular moment from vessel rotation offsets the pull of gravity on cell-scaffold constructs within it. Like the spinner flask, rotating wall bioreactors rely on diffusion for nutrient and gas transport but effectively remove gradients between the center and surface of scaffold constructs. Both bioreactor types, however, often result in different fluid shear forces experienced by cells at different positions in the scaffold. A third type of bone tissue engineering bioreactor is a perfusion bioreactor, in which medium is “perfused” through the porous scaffold to provide comparable nutrients, gas exchange, and fluid shear forces to cells throughout. Bioreactor culture of MSC-scaffold constructs seems to promote more consistent and complete osteogenesis than static culture. Continued research to recreate the natural fracture environment within a bioreactor may significantly enhance our understanding of the best pre-implantation conditions for MSC-scaffold constructs to augment fracture healing.

The information presented above provides only a glimpse of the complexity of MSC osteogenesis. Owing to intense public interest and focused research efforts, knowledge in this area continues to grow on a daily basis. There are numerous mechanisms to isolate and identify MSCs with osteogenic potential from most adult tissues. Clinical application of fresh and cryopreserved, autologous and allogeneic MSCs is rapidly becoming standard clinical fare. However, to date, there is limited standardization of adult MSC harvest, isolation, and expansion procedures. As presented above, in vitro and ectopic osteogenesis does not necessarily represent
orthotopic osteogenesis. Similarly, experimental reports surrounding MSC treatment outcomes vary widely. Rigorous preclinical investigations and clinical trials are necessary to solidify and condense the abundant research results into a viable clinical entity that is safe, effective, and reproducible. Harnessing the power of MSCs to augment fracture repair in companion animals may well be an achievable goal in the near future.