Perspective

Stem cells for the repair and regeneration of bone

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There continues to be a need for novel therapies directed at bone repair and regeneration in modern orthopaedics. Bone defects often arise secondary to trauma, nonunion, infection, tumor and periprosthetic osteolysis. The recognized shortcomings of autogenous bone grafting for the treatment of these conditions have led to a large field of investigation dedicated to the discovery of bone graft substitutes. This investigation has largely focused on osteoinductive growth factors [such as bone morphogenetic proteins (BMPs) and platelet-derived growth factor (PDGF)], osteoconductive scaffolds (such as calcium phosphate and other ceramics), or a combination of the two. Extensive preclinical investigation of these products has produced promising results. However, the results of clinical investigation in large-scale randomized trials have often fallen short of expectations, with relative efficacy similar to autogenous bone grafting being demonstrated.[1-3] This has led many scientists to explore more sophisticated techniques of tissue engineering for the repair and regeneration of bone. Among the most promising have been cell-based approaches, employing stem cell populations.

Most investigators have employed the use of mesenchymal stem cells (MSCs) due to the known ability of these cells for osteogenic and chondrogenic differentiation. Several studies have demonstrated that MSC therapy in combination with an osteoconductive scaffold or osteoinductive protein is capable of regenerating bone in preclinical models of fracture healing.[4-6] However, despite decades of preclinical investigation, there has been limited clinical translation of the animal studies which have demonstrated success with MSC therapy. There are a select number of case reports of MSC therapy for bone healing in humans in the literature, but no large-scale trials or prospective series have been reported. This limited clinical translation has been attributed to several different roadblocks including cost and safety issues associated with *ex vivo* expansion of MSCs, limited cell viability following transplantation, and lack of vascularity of MSC-loaded scaffolds. In addition, the results of preclinical studies have been mixed, with some authors reporting a lack of success with MSC therapy.[7]

More recently, several studies have demonstrated improved results of MSC therapy with genetically modified cells which produce osteogenic or angiogenic growth factors.[8] This technique uses MSCs which have been genetically modified to produce growth factors that enhance the repair and regeneration of bone. This strategy allows the local delivery of both cells which are crucial to the bone healing process and growth factors which are either osteoinductive or angiogenic. The genetically modified MSCs act as local factories producing the desired proteins in a local and sustained fashion. Several growth factors have been investigated with MSC gene therapy including, osteogenic proteins such as the BMPs and angiogenic proteins such as fibroblast growth factor (FGF) or vascular endothelial growth factor (VEGF).[8] In fact, the best results have been produced with MSCs genetically modified to produce both osteogenic and angiogenic growth factors, addressing both bone and blood vessel formation in a single strategy.[9,10] Once again, however, these positive preclinical results have been difficult to translate into clinical studies primarily due to the safety concerns, regulatory hurdles, and cost issues associated with gene therapy. At present, it seems unlikely that orthopedic surgeons will be using gene therapy for the treatment of bone healing problems in human patients anytime in the near future.

MSCs may not be the only type of stem cell capable of enhancing the repair and regeneration of bone. Our research group and others have recently reported on the use of a novel stem cell type for enhancing fracture healing, called endothelial progenitor cells (EPCs). EPCs represent a progenitor cell population of hematopoietic origin, with known ability to participate in angiogenesis.[11] It has been shown that EPCs home to sites of tissue ischemia, effect

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functional blood flow recovery in ischemic tissues, and enter the circulating system in response to trauma.[12,13] The effectiveness of EPCs at inducing angiogenesis in ischemic tissues is well documented in animal studies in the areas of cardiovascular disease, peripheral vascular disease, and stroke.[14-16] The remarkable success of EPC therapy in animal models of ischemia has led to clinical trials of EPC therapy for myocardial infarction and limb ischemia.[17,18] Given the known importance of vascularity for bone repair and regeneration, it is perhaps not surprising that several investigators have reported success with the application of EPCs to critical sized bone defects and non-union models in animal studies.[19-21] Therapy with EPCs has been shown to augment both fracture healing and local angiogenesis in animal models of non-union. More recent investigation has demonstrated possible synergism of combined MSC and EPC therapy for the repair and regeneration of bone, with the combined application of the two cell types demonstrating improved efficacy over the delivery of either cell type alone.[22] Given the multicellular nature of bone healing, these results are not entirely surprising. Both EPC therapy and combined MSC/EPC therapy represent promising new avenues of investigation in preclinical research on the repair and regeneration of bone.

While much has been discovered and investigated in regards to stem cell therapy for the repair and regeneration of bone, many questions remain unanswered. Most investigators have employed the use of stem cell therapy for bone regeneration based on the rationale that stem cells delivered to a site of desired bone healing would proliferate and differentiate into osteoblasts, contributing directly to the formation of bone. Recent investigation has questioned this hypothesis by failing to demonstrate that transplanted stem cells are directly responsible for the bone formation observed in response to stem cell therapy.[23] Rather, this investigation has suggested that stem cell therapy may exert its effects on bone healing by a variety of other ways including the secretion of growth factors which attract other bone forming cells or vasculature, anti-apoptotic effects, and immune modulation. It appears that these "trophic effects" of stem cell therapy may be more important than any direct contribution to the formation of bone. The recent demonstration that EPCs are effective at enhancing bone repair and regeneration would seem to further support this contention, given the fact that these cells are not commonly believed to differentiate into osteoblasts.

There continues to be a significant need for novel therapeutic approaches directed at the repair and regeneration of bone. Tissue engineering approaches which employ the use of stem cells remain a promising potential approach. However, prior to any significant clinical investigation, further preclinical research on stem cell therapy for the repair and regeneration of bone must be directed at clarifying many of the issues identified above. The ideal stem cell or combination of stem cells for enhancing bone healing must be identified. Certainly, the prospect of combining an osteogenic stem cell population, such as MSCs, and an angiogenic stem cell population, such as EPCs, represents a promising avenue for further research. The mechanisms by which stem cell therapy enhances bone repair and regeneration require further clarification. If in fact, their "trophic effects", such as growth factor secretion, prove to be the major mechanism by which they enhance bone healing, it may be more cost-effective and safer to identify those growth factors responsible for their positive effects and devise new therapies which employ those growth factors in an "off-the-shelf" strategy. However, the ability of cell-based therapies to provide continued growth factor secretion over time and respond to their microenvironment may be difficult to emulate with a single dose of growth factors. Even if stem cell therapy is never used in the treatment of humans for bone healing or only used in the most extreme cases, further investigation of stem cell therapy for the repair and regeneration of bone will help to advance our understanding of the cellular and molecular requirements for effective bone healing and continue to lead to novel therapies.

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