Effects of Endothelial Progenitor Cell Therapy on Diabetic Rat Fracture Healing

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Introduction: There has been substantial interest in tissue engineering strategies which employ the use of stem or progenitor cells for the treatment of bone defects and bone loss, representing a relatively novel field of study in orthopaedics. More specifically, a population of cells termed endothelial progenitor cells (EPCs) has been shown to promote vasculogenesis and osteogenesis [1], and their potential to provide therapeutic angiogenesis has been widely investigated in the fields of cardiovascular disease, peripheral vascular disease, and ischemic stroke [2, 3, 4]. Our group has previously shown that ex-vivo expanded EPCs can significantly enhance bone healing in a segmental rat defect model and that this effect is mediated, at least in part, through increasing local vascularity [5]. However, less is known about the therapeutic effect of EPCs in the context of systemic illnesses like diabetes. There is strong evidence that the biology of healing is significantly altered and impaired in the face of diabetes [6, 7, 8]. We sought to further investigate the properties of EPCs and their effectiveness in enhancing bone healing in diabetes. Our hypothesis was that the delivery of exogenous EPCs could enhance bone healing, even in the context of an impaired biological state such as diabetes. We studied this hypothesis using a rat segmental bone defect model wherein diabetes was chemically induced.

Methods: EPCs were isolated from rat bone marrow, cultured for 10-14 days in endothelial cell culture media, then harvested, seeded onto a gelfoam scaffold and re-implanted into either a healthy rat fracture model (n=10) or a diabetic rat fracture model (n=12). Control treatments consisting of gelfoam containing culture media only were also implanted in healthy rats (n=8) and diabetic rats (n=8). The segmental bone defect model consisted of creating a three-mm segmental bone defect in the diaphysis of the right femur, filling the defect with the indicated treatment, then stabilizing the construct with a plate and screws. In the diabetic group, diabetes was induced via intraperitoneal injection of 35mg/kg of streptozotocin two weeks prior to creation of the bone defect. Hyperglycemia was confirmed with glucometer testing on a regular basis throughout the study period. Rats were then sacrificed at 10 weeks and the femurs harvested and submitted for radiological analysis. Plain radiographs and microCT analyses were done on the samples. The study was approved by our Institutional Animal Use and Care Committee.

Results: When either healthy rats or diabetic rats were implanted with control-gelfoam, none (0/8) of the samples in either group healed. EPC treatment in healthy rats resulted in radiographic healing in 6/10 (60%) animals. In comparison, EPC treatment in diabetic rats resulted in radiographic healing in 5/12 (42%) animals (Table 1). Radiographs demonstrate a persistence of the bony defect, non-union, and absence of calcified material in groups with control-gelfoam treatment (Figure 1). In the EPC treated groups, healthy rats demonstrated abundant callous formation with the defect filling in with bone (Figure 1). Clinically, these samples also had adequate mechanical stability that the plate and screws could be removed. In comparison, when diabetic rats were treated with EPCs, there is callous formation; however the quality and quantity of bone appears inferior to that seen in healthy rats. MicroCT analyses confirmed the radiographic findings: for either healthy or diabetic rats treated with control-gelfoam, there is clearly no bone formation within the segmental defect (Figure 2). In the healthy animals treated with EPCs, there is abundant bone formation bridging the segmental defect with cortical as well as trabecular bone (Figure 2). In diabetic animals treated with EPCs, there is bridging callous; however, it is less organized and cortical remodeling is less apparent (Figure 2). This becomes clearer when focusing on the area of the segmental defect only (Figure 3). The morphology of the bridging bone in healthy, EPC-treated animals resembles that of native bone, with a clear remodelling of the cortex. Conversely, the morphology in diabetic, EPC-treated animals is less organized, with less cortical bone and more trabeculae (Figure 3).

Discussion: In this study, we have shown that implantation of EPCs in the segmental rat defect has a significant effect on enhancing bone healing. Whereas no control samples healed, we achieved a 60% union rate in the healthy, non-diabetic animals. Even when implanted in the diabetic animals, we report a union rate of 42%, suggesting that exogenous EPCs can, to some degree, rescue the pathological processes which impair fracture healing in the diabetic state. At the same time, the rescue potential of EPCs is limited, as demonstrated by the poorer quality of the regenerated bone within the defect.

Significance: We have demonstrated that implantation of EPCs into segmental bony defects can enhance bony regeneration. The treatment is not as effective in the context of diabetes, but still provides some additional regenerative potential. Continued research in this field could lead to tissue-engineered therapies for patients suffering from the morbidity of bony defects or non-union, including those patients with impaired healing due to systemic illnesses like diabetes.

Acknowledgments:

References: 1. T. Asahara, T. Murohara, A. Sullivan, M. Silver, R. van der Zee, T. Li and et al, "Isolation of putative progenitor

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Table 1. Radiographic results

![Figure 1. Representative radiographs from each group: A) Healthy rats implanted with control-gelfoam. B) Healthy rats implanted with EPC-gelfoam. C) Diabetic rats implanted with control-gelfoam. D) Diabetic rats implanted with EPC-gelfoam.](image-url)
Figure 2. Representative microCT volume-rendered reconstructions from each group: A) Healthy rats implanted with control-gelfoam. B) Healthy rats implanted with EPC-gelfoam. C) Diabetic rats implanted with control-gelfoam. D) Diabetic rats implanted with EPC-gelfoam