A Combined Treatment of Gene-Modified Stem Cells and Oxygenated Scaffolds for Scaphoid Nonunion with Avascular Necrosis

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Introduction: Avascular osteonecrosis (AVN) of the scaphoid bone occurs in the scaphoid non-union fracture when a bone fragment is deprived from oxygen and other nutrients thus becoming necrotic. This microenvironment presents unique challenges for bone regeneration. It was estimated that in the United States more than half a million carpal fractures and dislocations occur yearly, resulting in 3.5 million days lost from work. Although the great majority of scaphoid fractures heal uneventfully with conservative management, the incidence of scaphoid nonunion may range from 5 percent to as high as 12 percent [1]. It is believed that nonunion fractures occur in the scaphoid since after the fracture occurs a part of the bone is cut off from its vascular supply, resulting in avascular necrosis. Currently available techniques are not optimal and include internal fixation with bone grafting and vascularized bone grafting, often leading to co-morbidity. Other potential risks include infection, ankylosis, and persistent nonunion[1]. Stem cell therapy could be an attractive therapy for scaphoid nonunions. The potential of mesenchymal stem cells (MSCs) to regenerate bone tissue has been demonstrated in numerous studies, but MSCs require an osteo-inductive scaffold in order to promote bone formation in vivo. Another problem with insufficiency of oxygen supply can be found in scaffolds used for cell seeding. One option in overcoming hypoxic conditions within a tissue engineering scaffold is to increase the level of oxygen within the scaffold by using perfluorocarbons (PFCs) such as perfluorotributylamine (PFTBA). We propose to develop a stem cell therapy approach for the treatment of scaphoid nonunion with avascular necrosis. We hypothesize that the implantation of BMP-engineered MSCs suspended in PFTBA oxygen-enriched matrix will induce fracture repair in a scaphoid nonunion model. Our hypothesis is based on three sub-hypotheses: 1) the addition of PFTBA will enable the survival of the cells in the hypoxic environment of the fracture till neovascularization occurs [2]; 2) MSCs secret angiogenic factors and will induce angiogenesis at the implantation site [3]; 3) BMP is also known to be an indirect inducer of angiogenesis and will synergize revascularization of the fractured bone [4].

Methods: All procedures described in this study were approved by the Institutional Animal Care and Use Committees. A micro-Computed Tomography (uCT) scan was performed of a rat’s radiale bone to confirm the similarity of the rat osseous structure to the human scaphoid bone. A radio-opaque contrast agent was injected to the rat’s vessels, followed by uCT scan to evaluate the micro vascularization around the radiale bone. Next, a circular defect (1.8 mm in diameter and 1.8 mm in depth) in a rat radiale bone was created. Ten million constitutively BMP2 expressing MSCs under tet-off control were suspended in hydrogel (Fibrin Gel) supplemented with 10% PFTBA and implanted in the defected bone void. For controls, we used cells suspended in the same gel without PFTBA in the ectopic site in the same animals. Cell survivals in bone void and ectopic site were monitored 2, 3 and 4 weeks post-surgery.
using bioluminescence imaging. For each time point, animals were sacrificed and histology (H&E and MTC) were performed. Bone formation 42 days post-surgery were performed using uCT analysis. Next, to translate to a large animal, we examined the potential of using the pig model for scaphoid nonunion with avascular necrosis. CT scans were taken from pig forelimb and similarities with radiale bone and vascularization were analyzed.

**Results:** Anatomical correlations between the human scaphoid to the rat’s radiale bone are shown in Figure 1. The results from bioluminescence imaging shows much higher number of cell survival in 10% PFTBA compare to cells implanted only with fibrin gel at ectopic site. 2D slice and 3D reconstruction showed new bone highlighted with red in Figure 2 which demonstrated new bone formation at the radiale. PFTBA supplementation significantly increased structural parameters of bone in radiale bone defects. The anatomy, bone and vasculature, of the human wrist correlate closely to pig forelimb shown in Figure 3.

**Discussion:** The rat’s radiale is very similar to the human structure and can be used for optimization of BMP-engineered MSCs suspended in PFTBA-enriched matrix. Our results showed a potential of using PFTBA as a vehicle for longer cell survival at the sites that lacks oxygen and vascularization. For translational purpose, the pig radial carpal bone is similar to the human scaphoid, allowing us to use the pig as a model of scaphoid non-union fracture.

**Significance:** These fractures are especially challenging since they involve very limited blood supply most likely leading to avascular necrosis. The success of this project could lead to a novel therapy for numerous patients suffering from scaphoid fractures that do not heal.

![Figure 1: Anatomical correlations between the human scaphoid to the rat’s radiale bone. An illustration of fractured human scaphoid bone (A). 3D reconstruction of uCT scan, of an rat paw, after perfusion with a radio-opaque agent. The radiale marked with green, blood vessels marked with red (B). The isolated radiale and feeding collateral artery marked with red (C).](image)
Figure 2: BMP over expressing MSC were implanted, according to the protocol depicted above. MicroCT scanning was performed 42 days later. 2D slice and 3D reconstruction showed new bone highlighted with red.

Figure 3: Pig forelimb was harvested and perfused with a radio-opaque agent (microfil). Blue arrows indicate the collateral artery to the Radial Carpal (Scaphoid bone). Orange arrow indicates vessels feeding the Intermediate Carpal (Lunate) and the 2nd Carpal bone (Capitate).

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