Synthetic Oxygen Carriers Enhance Bone Defect Regeneration Induced by BMP-Expressing Mesenchymal Stem Cells

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INTRODUCTION:

MSCs can differentiate into the osteogenic, adipogenic, chondrogenic and tenogenic lineages, and therefore are promising candidates for skeletal tissue regeneration [1]. Combining MSCs and Bone Morphogenetic proteins (BMPs) has been shown to promote fracture repair and rapid bone formation in vivo [2]. It is also known that osteogenesis is highly dependent on vascularization and oxygen supply [3]. Moreover, hyperbaric oxygenation therapy that elevates the oxygen levels in tissues was found to accelerate bone formation induced by recombinant human BMP-2 protein [4].In tissue engineering applications, cells are seeded within a biodegradable scaffold, which could reduce oxygen availability, thus limiting the osteogenic effect. We **therefore hypothesized that oxygen-enriched scaffolds would enhance engineered MSC-based bone formation, in vivo.** In order to test this hypothesis we have utilized the perfluorocarbons synthetic oxygen carriers as a way to increase oxygen availability in hydrogels.

MATERIALS AND METHODS:

Tet-off BMP2 MSCs (C3H10T1/2 MSC line that overexpress the BMP-2 gene under Tetracycline regulation [2]) and Tet-off BMP2 Luc/GFP (Tet-off BMP2 MSCs that were genetically engineered to constantly express both luciferase and GFP reporter genes) were cultured to near confluency, when they were tripsinized and counted. Fibrin gel (Tisseel, Baxter, Germany) was prepared according to manufacturer protocol. Aliquots of cells (one million Tet-off BMP2 cells or 3 million Tet-off BMP2 Luc/GFP cells) were resuspended in 50ul of fibrin gel. Five or 10% (v/v) Heptacosafluorotributylamine (PFTBA, Sigma) was added to the hydrogels just before implantations. The cell-hydrogel mixture was injected subcutaneously (SC) in 8 weeks old female C3H/HeN mice. Mice were sacrificed after two weeks, and implants were harvested and fixed in 4% formalin. In another set of experiments, a 2.5-mm long non-union defect was made in the mice radius bone. One million tet-off BMP2 MSCs were suspended in 15ul fibrin gel. PFTBA (10%) was added to the gel-cell mixture that was subsequently implanted into the bone defect. As a control, unsupplemented cell-gel mixtures were implanted in similar defects. The limbs were harvested after two weeks. In order to quantify bone formation in the ectopic implants and in the bone defects, they were scanned in a high-resolution uCT system (uCT 40, Scanco). Bone volume and density (for both ectopic bones and retrieved radii) and trabecular thickness (for radii only) were calculated based of the uCT scans. Tet-off BMP2 Luc/GFP cell viability in the ectopic implants was quantified using the Roper Chemiluminescence Imaging System (model LN/CCD-1300EB, Roper Scientific, Princeton Instruments, USA). The implants were also analyzed histologically.

RESULTS:

Micro CT-based analysis of ectopic bone formation revealed that 10% PFTBA supplemented hydrogels enhanced bone volume significantly, compared with un-supplemented controls (Fig. 1 a, b). Bone density was similar as evident from the bone mineral density results, and from histological analysis. Moreover, when Tet-off BMP2 Luc/GFP cells were implanted in PFTBA enriched fibrin gel, cell viability, deduced from the bioluminescence signal, was enhanced significantly compared to implants that did not contain PFTBA (Fig 1 c,d) . PFTBA has also effect on non-union bone defect regeneration. Bone formation and defect regeneration was noted both in the 10% PFTBA group and in the unsupplemented control (Fig. 2a), but bone volume (Fig. 2b), trabecular thickness (Fig 2c) and bone mineral density (Fig 2d) were elevated when PFTBA was added.

DISCUSSION:

We have shown, for the first time, that supplementation of synthetic oxygen carriers to BMP-2 engineered MSC implants enhances bone formation in vivo. This effect is correlated with the enhancement of post implantation cell viability that we noted in vivo. Moreover, our data implies that this cell-gel-PFTBA system can be utilized in order to promote bone defects regeneration, in a more rapid and efficient way. After only two weeks, the PFTBA supplemented cells yielded massive bone formation including more mature bone, as was evident from our uCT results. We envision that combining synthetic oxygen carriers with genetically engineered MSCs and novel biomaterials could promote bone formation in challenging situations, such as inducing interbody fusion in the intervertebral disc, which is an hypoxic environment, and also promote other tissue regeneration processes as in the myocardium.

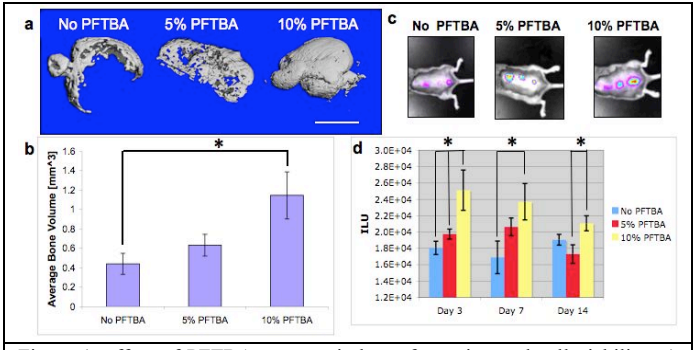

Figure 1: effect of PFTBA on ectopic bone formation and cell viability. a) Representative micro CT images of ectopic bone that was formed 14 days post implantation, bar=1mm. b) Supplementation with PFTBA enhanced bone volume, significantly (* P<0.05). n=13-14. c) Representative images of implants containing Tet-off BMP2 Luc/GFP MSCs at day 7, taken using the Roper Chemiluminescence Imaging System. d) Quantitative analysis of bioluminescence signal. Supplementation with PFTBA enhanced cell survival, significantly (* P<0.05, n=4-7).

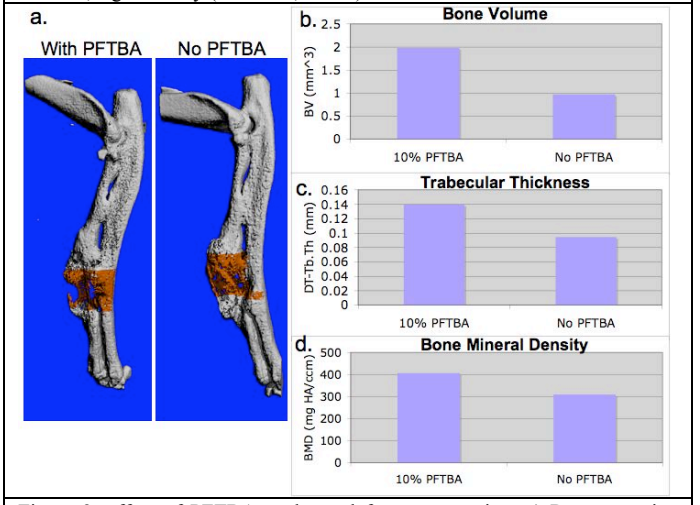

Figure 2: effect of PFTBA on bone defect regeneration. a) Representative micro CT images of radii bone that was regenerated 14 days post implantation. Bone formation in the defect site is highlighted in orange. b,c,d) Supplementation with PFTBA enhanced bone volume, trabecular thickness and bone mineral density, in the 10% PFTBA group compared with unsupplemeted controls.

References:

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