Orchestrated Stem Cell-based Regeneration (OSCeR) for Bone Tissue Engineering

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Statement of Purpose: Our recent studies have shown that using a biomaterial implant, usually in the form of a scaffold we can recruit autologous stem and progenitor cells. Such stem cells were found to be multipotent and have the potential to differentiate into various lineages; hence we call this phenomenon Orchestrated Stem Cell based Regeneration (OSCeR). Since, the naturally recruited cell numbers are low, we conducted preliminary studies using a cytokine erythropoietin (Epo) and found that they can recruit stem and progenitor cells. Since most tissue engineering scaffolds suffer from a common drawback of not being able to preserve the bioactivity of loaded cytokine, in our earlier study we developed a novel scaffold fabrication technique using protein microbubbles as porogen.[1] Such scaffolds were capable of preserving the bioactivity of cytokines and releasing them *in vitro* and *in vivo*. We hypothesize that by delivering Epo using tissue engineering scaffolds we can recruit and thereafter differentiate autologous stem cells into various lineages *in situ*. Since a recent study found that delivering angiogenic and osteogenic agents together can have a positive effect on osteogenesis,[2] we explored the potency of dual delivery of Epo along with bone morphogenetic protein-2 (BMP-2) in healing a critical size defect in an animal model.

Methods: *In vivo model:* OSCeR scaffolds fabricated as published earlier,[1] were loaded with cytokines Epo or Epo + BMP-2 and placed in acritical size defect in the mice cranium following a published procedure.[3] Defects without any scaffolds served as controls. Animals were monitored for 4 weeks. *Ex vivo imaging and histological analysis:* 24 hours prior to end of study, a hydroxyapatite (HA) specific near infrared dye – OsteoSense 800 (0.1 nM / 50 µl) was injected locally. After 24 hours the animals were sacrificed, cranium explanted, washed in PBS and imaged using a Kodak *In Vivo* imaging system. The cranium was then decalcified using 10% EDTA and embedded and stained for indicators of osteogenesis like osteocalcin.

Results: Based on fluorescence intensity of OsteoSense 800, after 4 weeks *in vivo* image analysis revealed that while there was very low indication of HA and almost no bridging of the defect in the untreated control, both the treatment groups showed signs of HA, implying osteoblast activity, and partial bridging of the defect (Fig. 1) In addition, based on osteocalcin expression (Fig. 2) it was found that Epo and Epo+BMP-2 had the highest osteocalcin expression that BMP-2 alone and was almost 20 times more than untreated control.

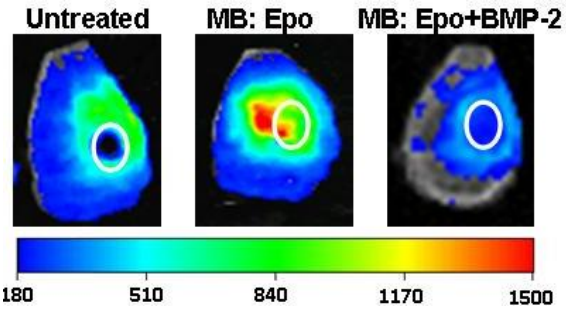

Fig. 1 Detection of hydroxyapatite around cranial implants after 4 weeks using OsteoSense 800 NIR dye

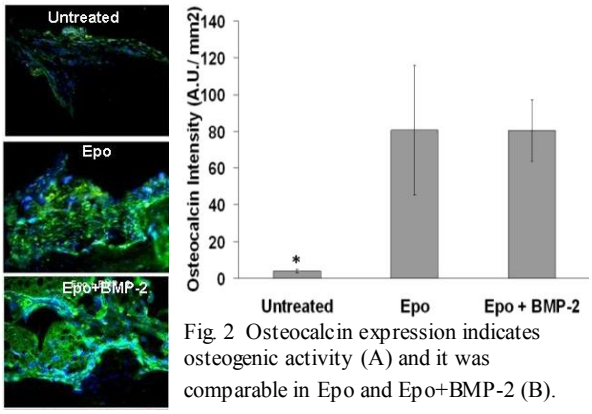

Fig. 2 Osteocalcin expression indicates osteogenic activity (A) and it was comparable in Epo and Epo+BMP-2 (B).

Conclusions: Building on our earlier studies which uncovered the phenomenon of OSCeR, we applied our novel bioactive cytokine releasing scaffolds to various clinically relevant conditions; one of them being bone tissue engineering. Based on the *ex vivo* HA imaging using the NIR dye, saw indications of HA production in both angiogenic Epo and dual delivery of Epo and BMP-2 which suggests osteoblast activity at the site. Osteocalcin expression at the site based on histology confirms this and also implies that the sign of osteogenesis is not just a surface feature but occurs inside the scaffold matrix as well. Further, our observation of collagen fiber deposition and osteopontin formation (not shown) indicates that as early as 4 weeks a dual delivery of angiogenic cytokine Epo and osteogenic BMP-2 can yield highly promising results. Although, longer term (8 week) studies are currently in progress, the early 4 week data suggests that using a novel scaffold that can preserve and deliver bioactive cytokines locally, either single or combination, can enhance the phenomenon of OSCeR and accelerate bone tissue regeneration and heal a critical size defect. This approach is currently being applied to various other clinically relevant conditions.

References:

1. A.M. Nair et al., Tiss Eng (C) 2010, 16:23-32.
2. Z.S. Patel et al., Bone 2008, 43:931-940
3. Z. Zhao et al.., J Dent Res 2007, 86:1207-1211.