Dose-controlled release of placental growth factor from a collagen-based scaffold promotes angiogenesis and enhanced bone defect healing

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INTRODUCTION: Regeneration of critically-sized bone defects remains a significant challenge. We have recently identified placental growth factor (PlGF) as a mechanically augmented gene which promotes angiogenesis at high doses and osteogenesis at lower doses [1]. Herein, we sought to functionalize a collagen-hydroxyapatite (CHA) scaffold to deliver PlGF in a dose-controlled manner, with a high burst release to promote angiogenesis followed by a lower sustained release to promote osteogenesis. Furthermore, we aimed to investigate the capacity of this scaffold to promote regeneration of a critically-sized defect in vivo.

METHODS: Recombinant human PlGF-2 (1 µg/mL) was incorporated into alginate microparticles (MPs; 0.5% w/v) and dispersed within CHA slurries pre-loaded with 0, 5 or 10 µg/mL PlGF-2 and freeze dried to form scaffolds termed 'PlGF-single', 'PlGF-dual low' and 'PlGF-dual high', respectively. In vitro evaluations were performed using ELISAs, matrigel angiogenic assays and osteogenic differentiation assays (n=3-4). 7 mm calvarial defects in Wistar rats were treated with scaffolds (n=7-8) and assessed using µCT after 28 days. Statistical comparisons were performed using ANOVA. Significance; $p<0.05$.

RESULTS & DISCUSSION: MPs were successfully incorporated within scaffolds. PlGF-single scaffolds showed a slow, consistent release whereas both PlGF-dual scaffolds demonstrated initial high burst release profiles followed by lower, sustained releases. When seeded with mesenchymal stem cells, PlGF-dual high scaffolds accumulated significantly more calcium compared to PlGF-free scaffolds ($p<0.001$). When added to human umbilical vein endothelial cells, elute from PlGF-dual high scaffolds promoted the formation of vessels with a significantly greater number of vascular junctions compared to PlGF-free groups ($p<0.001$). In vivo, PlGF-dual high scaffolds promoted significantly more bone formation compared to PlGF-free CHA scaffolds ($p<0.001$).

CONCLUSIONS: A dose-controlled release was achieved by combining the incorporation of PlGF directly within the scaffold, with the encapsulation of MPs, leading to a high burst release followed by a lower, sustained release. The harnessing of this dose-based effect allows for the delivery of pro-angiogenic and pro-osteogenic cues, key aspects of the regenerative process. This work provides a template for a mechanobiology-informed approach to regenerative medicine, by utilizing a therapeutic previously identified by leveraging the differential response of genes to mechanical loading [1].

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REFERENCES

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