Multi-functional gene-activated scaffolds for the repair of large volume defects in both healthy and osteoporotic bone

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INTRODUCTION: Non-union bone fractures caused by trauma and complicated by comorbidities such as osteoporosis, do not heal without interventions, which are frequently limited by donor site morbidity, infection risk, and availability, hence the increasing need for alternatives (1). Gene-activated scaffolds provide an opportunity to enhance bone repair by sustained delivery of therapeutic genes from biomaterial scaffolds providing a structural regenerative template (2,3). Within our research group, this strategy has previously been utilized to repair in vivo calvarial bone defects by delivery of osteogenic and angiogenic plasmid DNA (pDNA) and microRNAs incorporated into collagen-nanohydroxyapatite scaffolds (4–6). With a view towards treatment of severe bone defects, such as also in pathological context, the aim of the present study is to develop a gene-activated scaffold platform for bone repair in a complex in vivo weight bearing model by delivering multiple nucleic acid cargos, using multifunctional cell penetrating peptides, the glycossaminoglycan enhanced transduction (GET) peptide and a modified GET peptide (GET*), as delivery vectors (7). Specifically, we adopted a pro-angiogenic strategy for the repair of large volume defects in healthy bone using pDNA encoding chemokine stromal derived factor-1 alpha (pSDF-1α), and osteoporotic bone defects using antiimir-100 (targeting miRNA-100, which is overexpressed in serum, osteoblasts and osteoclasts isolated from osteoporotic patients) (8,9).

METHODS: In vitro optimisation and assessment of gene-activated scaffolds: A series of formulations of GET and GET* peptides were incorporated into 100% wt. collagen-nanohydroxyapatite (coll-NHA) scaffolds and assessed for pDNA delivery to mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs), and antiimir-100 delivery to MSCs derived from osteoporotic patients and ovariectomized (OVX) rats. In vitro assessment of gene-activated scaffolds was carried out using gene expression (PCR), metabolic activity, DNA content, protein expression (ELISA), angiogenic capacity (basement membrane assay) (n=3). In vivo assessment of gene-activated scaffolds: pSDF-1α activated scaffolds were assessed in an ex vivo chorioallantoic model for angiogenic potential. Following femoral defect surgery, gene free control scaffolds and pSDF-1α and antiimir-100 activated scaffolds were implanted in critically sized femoral defects into healthy and OVX rats, respectively. Femora were harvested 8 weeks after implantation and new bone formation was assessed by Micro-Computed Tomography (µCT) and histological assessment (H&E, Masson Goldner’s trichrome, TRAP, von Kossa) (n=8). Vascularization and formation of calcified scaffold was also quantified by image analysis, [The use of healthy and OVX female Sprague Dawley rats for a femoral defect procedure was approved by the RCSi Animal Research Ethics Committee under project number 202010014, and the Health Products Regulatory Agency under project authorization P909].

RESULTS: The GET* nanoparticles were significantly more effective than unmodified GET. GET* N/P 8 (ratio amine in vector: phosphates in nucleic acid) nanoparticles were the most effective formulation at delivering reporter pDNA and antiimirRNA to healthy and osteoporotic MSCs. pSDF-1α activated scaffolds significantly upregulated pSDF-1α expression in MSCs and MSC/EPC co-cultures. Furthermore, angiogenic parameters such as junction formation, and number and length of vessel-like structures were significantly increased in co-cultures in vitro and in the ex vivo model. Antiimir-100 activated scaffolds significantly downregulated miR-100 expression by 15-20-fold in healthy and OVX MSCs, demonstrating a 3-fold improvement on 2D results and other delivery vectors on the same scaffold. When the scaffolds were assessed in vivo, healthy rats, pSDF-1α activated scaffolds formed significantly more bone volume at 8 weeks, compared with gene free scaffold controls (Fig 1). Furthermore, histomorphometry revealed that pSDF-1α activated scaffolds significantly increased vascularization and calcified bone formation. In OVX rats, antiimir-100 activated scaffolds significantly increased bone formation at 4 weeks compared with gene free controls, as well as at 8 weeks post-implantation compared with baseline measurements taken at week 2 (Fig 2). Histological analysis demonstrated a significant increase in vascularity as well as mineralized bone deposits.

DISCUSSION: We identified a specific nanoparticle formulation (GET* N/P 8) which was most effective for both pDNA and antiimirRNA delivery, demonstrating the versatility of GET* nanoparticles. When used for gene delivery in the scaffolds, SDF-1α activated scaffolds enhanced angiogenesis synergistically with co-cultures of MSCs/EPCs, both cell types resident in bone marrow that are instrumental to bone repair. This pro-angiogenic approach proved successful in vivo, comparing favorably with reports in the literature, which describe good outcomes of SDF-1α delivery, albeit using riskier, more expensive delivery systems, or implantation of pre-transfected cell sheets. Similarly, highly significant inhibition of miR-100 expression by antiimir-100 activated scaffolds in OVX MSCs translated to enhanced bone formation in osteoporotic defects in vivo. Although the exact role of miR-100 in osteoporosis is compromised, this comparison of these results with reports in the literature suggests that miR-100 may act via the BMPR2 osteoblastic pathway. Encouragingly, the antiimir-100 activated scaffold also increased vascularization within the defect, indicating a potential role of miR-100 in angiogenic pathways such as VEGF. The outcomes of this study were particularly encouraging, as existing in vivo studies in OVX animals of miRIR delivery tend to be based on viral delivery systems, or in non-weight bearing defect models which arguably present a lesser challenge.

SIGNIFICANCE/CLINICAL RELEVANCE: A non-viral scaffold-based delivery system capable of delivering therapeutic cargoes to enhance tissue regeneration in a load bearing defect in diseased bone tissue is a significant advance in bone tissue engineering research. The positive outcomes of delivery of multiple cargo types using the same delivery platform demonstrated versatility and potential for a myriad of other tissue engineering applications.


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IMAGES: Fig I. Calcified bone (shown using Masson Goldner’s trichrome stain) and bone volume (%) were significantly increased by gene-activated scaffolds (pSDF-1α activated scaffolds in healthy rats, antiimir-100 activated scaffolds in OVX rats). Significance p<0.05*, p<0.01**. Scale bar = 1000 μm.