Development of a Highly Efficient PTH-1-34-activated Scaffold to Repair Large Bone Defects

Austyn R. Matheson 1,2,3, Joanna M. Sadowska 1,2,3, Rachael N. Power 1,2,3, Katie McCormick 1,2,3, Eamon J. Sheehy 1,2,3, Hyab Mehari Abbraha 1,2,3, Kian F. Eichholz 2, Pierluca Pitacco 1, Gang Chen 1,2,3, Fergal J. O’Brien 1,2,3

1 Tissue Engineering Research Group, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland. 2 TCBE, Trinity College Dublin (TCD), Ireland. 3 Advanced Materials and Bioengineering Research Centre, RCSI and TCD, Dublin, Ireland. email: austynmatheson@rcsi.com

INTRODUCTION: Addressing large-volume bone defects, which frequently result in delayed or non-union fractures, remains a substantial orthopedic challenge, particularly with bone compromised by osteoporosis. Our lab has developed collagen-nanohydroxyapatite (coll-nHA) scaffolds with proven efficacy for in vivo bone tissue repair applications (1,2). Enhancing these scaffolds with genetic cargoes, such as plasmid DNA (pDNA) encoding osteogenic factors, presents a promising approach to amplify their regenerative capabilities and mend larger critical-sized bone defects (3). While the scaffold offers essential structural support for the regrowth of bone tissue, the introduction of therapeutic pDNAs can activate specific osteogenic genes in native cells, with fewer unintended side-effects than observed when delivering high doses of growth factors such as bone morphogenetic protein 2. In this study, we examined the non-viral delivery of parathyroid-hormone-1-34 (PTH-1-34), known in its recombinant form as teriparatide, a potent FDA-approved osteoanabolic agent that has previously been shown to augment bone mass and osseointegration when loaded onto biomaterials (4,5). Therefore, the goal of this study was: 1) to develop a novel PTH-1-34-gene-activated scaffold system by complexing PTH-1-34 to nanohydroxyapatite (nHA) nanoparticles within coll-nHA scaffolds and 2) to demonstrate an enhanced bone repair response following implantation of the scaffold in a load-bearing critical-sized femoral defect.

METHODS: 1) Physicochemical characterisation of PTH-1-34 nanocomplexes: PTH-1-34 plasmid and nHA nanoparticles (NPs) were previously developed for gene delivery (2), were complexed and characterized (size, polydispersity index, zeta potential by dynamic light scattering). The functionality of NPs delivered to rat mesenchymal stem cells (MSCs) in monolayer was evaluated in terms of transfection efficiency, gene expression (PCR), and mineralization (Ca2+ quantification - (Ca2+ - quantification) of cells maintained in growth (GM) & osteogenic media. 2) Development of PTH-1-34-activated scaffolds: The coll-nHA scaffolds were prepared using freeze drying techniques (5) followed by incorporation of nHA-PTH-1-34 NPs at 2 µg of pDNA (2). The in vitro evaluation with MSCs included the biological assessment of metabolic activity, DNA content, cell distribution (H&E staining), transfection efficiency, expression of osteogenic genes (COL1, RUNX2, OCN) and mineralization (Ca2+ quantification - (Ca2+ - quantification) of cells. The scaffold was implanted into 5 mm plated femoral defects; bone volume and density were assessed by µCT at 4 and 8 weeks post-surgery. The samples were harvested at 8 weeks and assessed histomorphometrically using H&E to evaluate cell infiltration, and Masson’s Goldner Trichrome to quantify blood vessel formation.

RESULTS: 1) PTH-1-34 nanohydroxyapatite complexes were developed as a non-viral gene therapy at sizes of 1.78±0.32nm, with low polydispersity index (0.46±0.06), and a negative charge (-12.08±24.18mV). PTH-1-34 NPs effectively transfected MSCs increasing PTH-1-34 expression across multiple timepoints in comparison to non-transfected and lipofectamine (LF) controls (Fig. 1a), demonstrating the efficacy of nHA for non-viral gene therapy, an effect desired in the administration of PTH-1-34 which has optimal effects with transient dosing. PTH-1-34 NPs increased osteogenic responses of MSCs evident by 20-fold increase in calcium deposition (Fig. 1d), upregulated genes (COL1, RUNX2) (Fig. 1b) and pro-angiogenic response (ALP, VEGF) (Fig. 1c), as described previously (6). 2) The PTH-1-34 activated scaffolds supported transient transfection up to day 14 upregulating PTH-1-34 beyond timepoints noted in 2D (Fig. 2b). Again, early osteogenic markers were upregulated (Fig. 2a) with trends in increased late markers (OCN), leading to increased calcium deposition for PTH-1-34-activated scaffolds (Fig. 2c). When implanted in vivo, host cells infiltrated PTH-1-34 scaffolds and pDNA-free control scaffolds to a full thickness depth (H&E staining), demonstrating excellent cell migration and integration of scaffolds with the host tissue. Compared to pDNA-free scaffolds, PTH-1-34 scaffolds enhanced blood vessel infiltration (Masson’s Goldner) (Fig. 3a), increasing the number of vessels (Fig. 3b) and new bone formation (Fig. 3c). Notably, the in vivo assessment showed that the PTH-1-34 activated scaffolds promoted bone healing in femoral defect (Fig. 3c).

DISCUSSION: This study underscores the transformative therapeutic potential of a novel, PTH-1-34 gene-activated scaffold system. The PTH-1-34 NPs exhibited excellent functionality, not only upregulating PTH-1-34 expression in a transient manner, but also boosting the osteogenic repair responses of stem cells. The scaffold system effectively delivered upregulated PTH-1-34 to MSCs in vitro, elevating the expression of early osteogenic markers, promoting mineralization, and increasing calcium production across early and later timepoints. Of principal significance, the implantation of PTH-1-34 activated scaffolds led to notable increases in bone regrowth in addition to enhanced vessel formation, while showcasing excellent tissue integration and cell infiltration. Overall, the study confirmed that the PTH-1-34-scaffold system fully regenerate large osteonecrosis and bone regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: The scaffold-based system for the delivery of therapeutic PTH-1-34 genes, conceived in this study, holds immense promise as a foundation for bone regeneration, not in typical bone repair scenarios but importantly for patients confronting challenging and hard-to-heal fractures, like those in osteoporosis. Our work demonstrates, for the first time, the feasibility of deploying non-viral gene delivery of PTH-1-34 via scaffold-based approaches which opens the door to new possibilities to address the complex challenge of healing osteoporotic bone fractures.


ACKNOWLEDGEMENTS: ReCaP: European Council Advanced Grant (788753), National Science Foundation (NSF) and Science Foundation Ireland (SFI) (NSF_17_US_3437), ADMIRE Marie Skłodowska-Curie-Action-Cofund (EU Horizon 2020: 945168, SFI: 12/RC/2278_2), National Sciences and Engineering Research Council of Canada (PDF-578347-2023), The ON Foundation (ON Kick-Starter Grant Bone)

Figure 1. a) PTH-1-34 NPs upregulated expression of PTH-1-34 to day 7, and both the b) osteogenic (COL1A1, RUNX2) and c) angiogenic (VEGF, ALP) responses to day 14, and d) a 20-fold increase in calcium production.

Figure 2. a) PTH-1-34 activated scaffolds effectively transfected MSCs, significantly elevating osteogenic markers and b) osteogenic gene expression, c) calcium deposition.

Figure 3. a) PTH-1-34 scaffolds accelerated healing with greater cell infiltration, b) vascularization, and c) bone repair in a femoral defect model. Scale=200µm.