Development Of A Gene-Activated Composite Scaffold For Load-Bearing And Controlled Regeneration Of Zonal Articular Cartilage Properties

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Email: Tomhodgkinson@rcsi.com **Disclosures:** F.J. O'Brien – (3A, 3B, 5, 6, 7A, 8)

Introduction: Articular cartilage (AC) repair is a significant clinical challenge with limited effective treatment options. In part, this is due to both the complex, zonal structure, and specialized composition of cartilage extracellular matrix (ECM) and the limited reparative capacity of resident cells [1]. Advanced biomaterials have the potential to improve cartilage regeneration through provision of instructive cell environments, but engineering cartilage-like biomaterials has proven challenging. Biomaterials can be designed with specific physicochemical properties that aid in control of cell phenotypes and deliver regenerative genetic cargo, but material stiffnesses that drive cell regeneration of articular cartilage (kPa range) are vastly lower than those required to support joint load-bearing (MPa range). Hydrogels formed of naturally occurring polymers have great promise to improve cell phenotypes and regeneration of articular-like ECM but typically have insufficient mechanical properties for load bearing. Through precision deposition and integration of biomaterials, additive manufacturing offers a solution by allowing the engineering of regenerative constructs with the potential to simultaneously recreate the zonal mechanical properties of cartilage, while providing softer zone-specific cell regenerative environments [2]. Therefore, by integrating melt electrowriting (MEW) and fused deposition modelling (FDM), this study first aimed to engineer a cartilage regeneration construct combining regenerative hydrogels and reinforcing microfibrous scaffolds, providing zonal cell environments and load-bearing properties. Next, to improve the repair potential of resident cells, this biomaterial was functionalized for the non-viral delivery of transcription factors targeting reversal of age-associated DNA methylation, restoring youthful regenerative phenotypes. This approach allows optimization of regenerative and load-bearing biomaterial components to support rapid restoration of tissue functionality, while targeting repair-limiting cell processes to enhance cartilage regeneration.
Methods: To engineer regenerative hydrogels, methacrylated hyaluronic acid (MeHA) and gelatin (GelMA) were synthesised in-house and chemically characterised. MeHA/ GelMA blends were produced containing LAP photoinitiator. The rheological properties of pre-gel solutions were assessed via amplitude and shear sweep tests. Gels were photocrosslinked and their mechanical (compressive; tensile), swelling, mesh size and degradation properties determined in physiologically relevant solutions. Hydrogel formations were selected with a range of physicochemical properties for biological testing through incorporation of either human articular chondrocytes (hACs) or human bone marrow derived mesenchymal stem cells (hMSCs). The impact of gel formulation on cell viability and phenotype was assessed. Following this, cell-laden hydrogels were cultured for 28 days in either chondrogenic (+TGFβ3) or expansion media (-TGFβ3) and chondrogenic ECM production was assessed through biochemical and (immuno)histological assays. For development of corresponding MEW/FDM reinforcing scaffolds, candidate designs were fabricated in polycaprolactone (PCL) based on required mechanical properties of the superficial, intermediate, and deep cartilage zones. Scaffolds were characterized by scanning electron microscopy and their mechanical properties determined through tensile and compressive testing. Finally, best performing MEW/FDM zonal scaffolds were assembled into a continuous construct and interpenetrated by corresponding cell-laden zonal hydrogels to create a layered but continuous regenerative construct. For gene delivery, glycosaminoglycan binding-enhanced transduction (GET) peptide was optimized for non-viral delivery to hACs and hMSCs. Transgene expression was controlled through a 4-HT ON expression vector and the effects of expression on assessed in chondrogenic and expansion culture.
Results: Chemical characterisation demonstrated high batch-to-batch consistency in methacrylation of hyaluronic acid and gelatin. Rheologically, pre-gel solutions possessed viscosity suitable for interpenetration of MEW/FDM reinforcing scaffolds (Fig. 1A). After photocrosslinking, formulations varied significantly in their compressive properties, including Young's moduli (Fig. 1B, 1C). Hydrogels across physiologically relevant stiffness ranges were selected for biological testing [3], with cell culture analysis demonstrating excellent hAC and hMSC viability for all formulations following encapsulation. Formulation mechanical and molecular composition influenced cell morphology, ECM production and distribution. Hydrogels supported chondrogenic differentiation over 28 days culture in the presence of TGF-β3, showing differential chondrogenic gene and protein expression. MEW/ FDM scaffold fibre properties and architectures could be effectively controlled by manufacture parameters to produce uniform scaffolds greater than 30 layers thick. Fibre properties and architectures were found to influence compressive and tensile properties of scaffolds, with design dependent levels of anisotropy. Compressive moduli of scaffolds was in the range of previously measured stiffnesses of cartilage tissue zones. MEW/ FDM scaffold design allowed their efficient incorporation into corresponding hydrogels to create a composite, mechanically reinforced biomaterial. For non-viral gene delivery, the GET peptide was optimized for delivery to hACs and hMSCs, producing nanoparticles with diameters of approximately 120 nm with a weak positive charge. In both hACs and hMSCs, GET was highly cytocompatible, producing high transfection efficiency, low cytotoxicity and high reporter (GFP; *Luc*) expression compared to lipofectamine controls. Transgene expression was tightly controlled by the developed 4-HT ON system (Fig.1D,1E) in culture and impacted healthy chondrogenic gene expression and ECM production.
Discussion: This study details the development of a composite biomaterial strategy for cartilage repair that is designed to support both cell-led regeneration and tissue loading. Through independent variance of hydrogel formulations and microfibrous scaffold designs, control of both cell phenotypes and mechanical properties was possible with greater precision. In particular, by varying MeHA to GelMA ratio it was possible to influence cell morphology, gene expression and ECM deposition. This approach is promising for the development of zonal biomaterials capable of replicating both the zonal cell phenotypes and the mechanical properties of healthy cartilage. Functionalisation of these biomaterials for non-viral gene delivery improved the expression of regenerative markers, highlighting the promise of combining instructive biomaterials and therapeutic gene delivery for enhancing cartilage repair.
Significance/Clinical Relevance: Regenerative hydrogels have great potential as biomaterials to improve cell-mediated cartilage regeneration but their clinical use is limited by the mechanical demands of articular cartilage. In this study, a composite material design created through precision additive manufacturing techniques allows for the optimization of functionalized biomaterial cell regenerative properties and loading characteristics. This approach increases the utility of promising regenerative hydrogel formulations in mechanically-loaded regions of cartilage and allows replication of both zonal cell phenotypes and mechanical properties, refining the composition, structure and functionality of the tissue produced.
REFERENCES:[1]Hunter et al.(2020) Lancet.396:1712. [2]O'Shea et al. Biomater. Sci. 10:2462. [3]Guilak et al.(2018) Matrix Biol. 71:40.
ACKNOWLEDGEMENTS: This work was funded by Science Foundation Ireland Grant number:21/PATH-S/9306.
IMAGES AND TABLES:

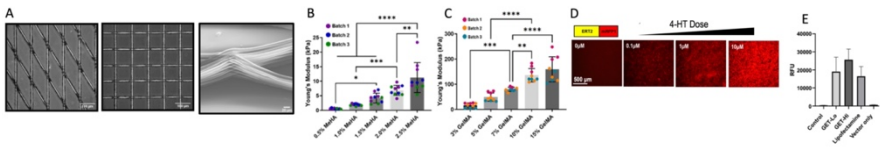

Fig. 1 (A)SEM images of MEW fibre designs. Compressive moduli of (B) MeHA and (C) GelMA hydrogels formulations (D) Sensitivity of 4-HT ON expression system (E) Luciferase expression in hACs following GET delivery.