In vivo Validation of a Continuous Gradient Porous Scaffold for Osteochondral Defect Repair in a Rabbit Model

Kyra W.Y. Smith[1,2], Soheila Ali Akbari Ghavimi[2], Stephanie Logterman[2], Paul M. Gehret[1,2], Elisa Capuana[3], Gioacchino Conoscenti[3], Valerio Brucato[3], Vincenzo La Carrubba[3], J. Todd Lawrence[2], Riccardo L. Gottardi[1,2]
[1]University of Pennsylvania, Philadelphia, PA, [2]Children's Hospital of Philadelphia, PA, [3]University of Palermo, Italy

Statement of Purpose: Osteochondral defects are a major health concern affecting 60% of patients who undergo knee surgery[1] and are characterized by damage to both articular cartilage and subchondral bone. Current standards of care do not achieve regeneration, so tissue engineering represents an exciting alternative, but a key hurdle is the tendency of engineered cartilage to ossify.[2,3] To overcome this limitation, we developed a porous osteochondral scaffold in which specific pore sizes are tailored to promote chondrogenesis on one side and osteogenesis on the opposite side. The scaffold is made of Poly(L-Lactide) (PLLA) and the pore dimensions vary along a continuous gradient (~70µm diameter, pro-chondrogenic[4] to ~200µm diameter, pro-osteogenic[5]) to avoid delamination. We used a biphasic bioreactor[6] for parallel chondrogenic and osteogenic differentiation of each side of the osteochondral constructs which were then tested *in vivo* in an osteochondral defect rabbit model.

0Methods: Scaffolds were fabricated by thermally induced phase separation to form a continuous gradient of pore sizes along the sample height (Fig. 1A).[7] Cylindrical scaffolds (diameter=4mm, height=6mm) were uniformly seeded with 200K rabbit mesenchymal stem cells and placed in the biphasic bioreactor for osteochondral differentiation (Fig. 1B). The small pore side was perfused in the upper chamber with chondrogenic medium, and the large pore side was perfused in the lower chamber with osteogenic medium, both at a flow rate of 1.4µL/min. After three weeks, differentiation was assessed first by histology (Fig. 1D,E). The top and bottom third of the constructs were separated and analyzed via RT-qPCR for chondrogenesis (Fig. 1F) and osteogenesis (Fig. 1G), or mechanical testing (Fig. 1C).[8] Bilateral osteochondral repair was performed on 21 female New Zealand White rabbits (3.5-4.5kg) for a total of 42 knees. The experimental groups were: (i) empty defects (negative control), (ii) acellular scaffolds, (iii) non-differentiated MSC-seeded scaffolds, (iv) pre-differentiated engineered osteo-chondral constructs. In brief, the trochlea was exposed, a surgical drill was used to create a cylindrical osteochondral defect 4mm wide by 6mm deep, and the scaffold was press-fit into the defect with the large pore side facing the marrow cavity. After euthanasia at 3 months, the rabbits' knees were examined for gross appearance, integration, and repair, then excised for microCT and histology to assess differentiation and integration and for RNAscope to assess spatial distribution of gene expression across the repair.

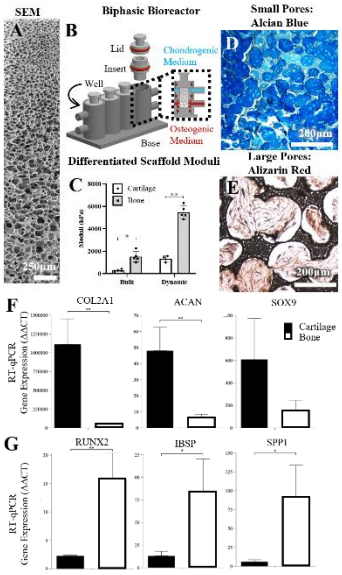

***Fig. 1: A)** SEM of pore gradient scaffold. **B)** Biphasic bioreactor. **C)** Differentiated scaffold moduli. **D)** Alcian blue for GAGs of chondral region. **E)** Alizarin Red for calcium of osseous region. **F)** Chondro- and **G)** osteogenic gene expression for both construct sides.*

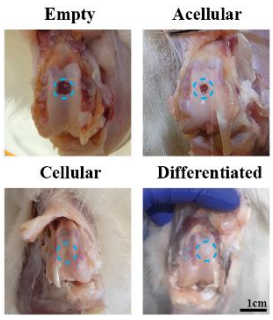

***Fig. 2:** Post-sacrifice gross appearance of scaffold integration and defect closure.*

Results: Pre-surgical characterization of the scaffold shows that when differentiated, there is a significant difference in both the bulk and dynamic moduli between the chondral and osseous regions of the scaffold, approaching native articular cartilage values. Strong chondrogenic phenotype was observed in the chondrogenic region (Fig. 1D,F), while strong osteogenic phenotype was observed in the osseous region (Fig. 1E,G). At the end of the three-month healing period, all rabbits were ambulating normally and showing no signs of pain or distress. Healed osteochondral defects show complete defect closure and integration in cellular and differentiated groups, and non-closures in most empty and acellular groups (Fig. 2). Ongoing examination by microCT shows both cartilaginous and bone tissue structures present in healed defects, proving successful biphasic morphologies. Our *in vivo* results confirm *in vitro* results regarding histology and gene expression for distinct chondrogenic and osteogenic differentiation.

Conclusion: Our continuous pore gradient scaffold avoids the risk of delamination common with biphasic models and promotes dual chondro- and osteogenic differentiation to align with the osteochondral bilayer in the knee. Construct maturation within the dual-flow bioreactor allows the simultaneous differentiation of each tissue type within a monolithic structure ready to be implanted. Application of the scaffold to an *in vivo* rabbit model confirms success of scaffold integration and osteochondral tissue healing suggesting significant promise for clinical translatability.

References: [1]Graham P. Orthop. Nurs. 2017;36:237-239. [2]Howel M. Curr. Rev. Musculoskelet. Med. 2021;14:60-66. [3]Saka N. Regen. Med. Res. 2019;7:1-8. [4]Nava M. J. Appl. Biomater. Funct. Mater. 2016; 14:223-229. [5]Lin T. Int. J. Mol. Sci. 2019;20:326-347. [6]Ianetti L. PLoS One. 2016;11:1-25. [7]Gottardi R. ORS Proceedings. 2016. [8]Patel J. Tissue Eng. Part C Methods. 2019;25:593-608.