Scaffold pore size controls chondrogenic differentiation of human mesenchymal stem cells and cartilage formation *in vitro* and *in vivo*

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Statement of Purpose: Scaffold pore size plays an important role in cell attachment, migration, and differentiation. It has been reported that pore size affects chondrocyte behavior, but optimal pore size is inconclusive among different scaffolds. Furthermore, the effect of pore size on chondrogenic differentiation of stem cells remains unclear. Previously, we demonstrated that two-dimensional nano-fibrous (NF) poly-L-lactide (PLLA) matrix promoted human bone marrow-derived mesenchymal stem cell (hMSC) commitment along the chondrogenic route [1]. Similarly, our three-dimensional (3D) porous NF scaffolds also supported chondrogenic differentiation of hMSCs *in vitro* [1]. In this study, we compare the chondrogenic differentiation of hMSCs on NF scaffolds with small pore size (125-250 μm) or large pore size (425-600 μm) both *in vitro* and *in vivo*.

Methods: NF PLLA scaffolds were fabricated using thermally-induced phase separation. To control pore size, a sugar porogen template was created with sugar spheres 125–250 μm or 425–600 μm in diameter, with heat treatment for interconnection. Human MSCs (2.5×10^5 cells) were seeded into each scaffold (3.6 mm in diameter and 1 mm in thickness). To induce chondrogenesis, cell-seeded scaffolds were maintained in chondrogenic medium with 10 ng/mL TGF-β1 (Peprotech, Rocky Hill, NJ) for up to 4 weeks. Scaffolds were then implanted subcutaneously into nude mice for 8 weeks.

Results: To examine the effect of pore size, we fabricated small (125-250μm) (Fig. 1A) and large (425-600μm) (Fig. 1B) pore scaffolds with NF features (Fig. 1C-D). When seeded with hMSCs, cells were able to adhere to small (Fig. 1E) and large (Fig. 1F) pores with no significant difference in cell seeding efficiency, shown by DNA quantification 24 hours after seeding (Fig. 2A).

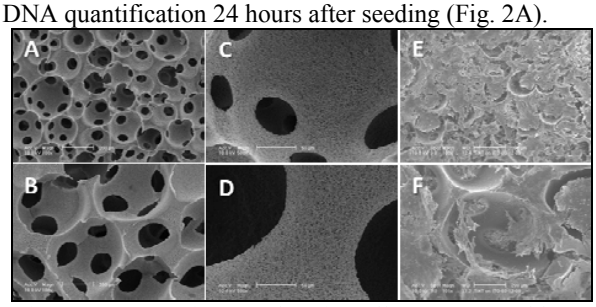

Figure 1. SEM micrographs of small (A, C, E) and large (B, D, F) pore size scaffolds, showing interconnected pore structure and nanofibers. (E,F): 24h after seeding scaffolds with human MSCs.

Following chondrogenic culture with 10ng/ml TGF-β1, hMSCs secreted a significantly higher amount of glycosaminoglycan (GAG) when cultured on small pore scaffolds compared to large pore scaffolds after both 2 weeks and 4 weeks (Fig. 2B). Small pore constructs had significantly higher collagen type II gene expression (Fig. 2C) and significantly lower collagen type I expression (Fig. 2D) after 2w chondrogenic culture, revealing enhanced chondrogenic differentiation on small pore scaffolds *in vitro*.

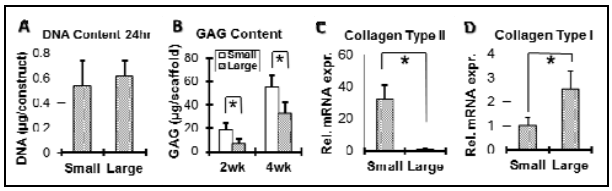

Figure 2. (A) DNA content at 24h. (B) GAG content at 2w, 4w. (C,D) Collagen II & I gene expressions at 2w.

In order to determine *in vivo* response, constructs were implanted subcutaneously for 8w following 4w *in vitro* chondrogenic culture. Consistent with *in vitro* results, small pore scaffolds promoted cartilage formation with typical cartilage morphology, depicted by hematoxylin and eosin (H&E) staining (Fig. 3A). Small pore scaffolds even supported ectopic endochondral ossification due to the advantageous cartilage template (Fig. 3A, arrows). However, large pore scaffolds succumb to fibrous tissue invasion after 8w, preventing chondrogenic phenotype maintenance (Fig. 3B). In addition, positive Safranin O staining for glycosaminoglycan (GAG) matrix was only achieved with small pore architecture (Fig. 4C-D). We hypothesize that smaller pores may have enhanced hMSC aggregation for chondrogenic commitment and prevented vascular ingrowth.

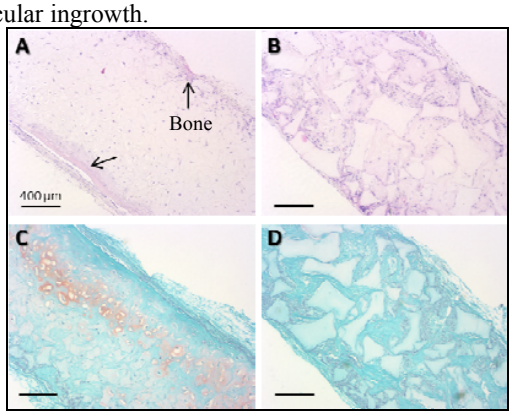

Figure 3. (A,B) H&E and (C,D) Safranin O staining of small pore (A,C) and large pore (B,D) scaffolds following 8w mouse subcutaneous implantation.

Conclusions: We showed that a small pore size (125-250μm) enhanced chondrogenic differentiation of hMSCs *in vitro* compared to a large pore size (425-600μm) and improved cartilage formation following mouse subcutaneous implantation. This study provides a useful method to control the cartilage regeneration process with highly designed pore architecture of porous nanofibrous scaffolds. Future work will explore the mechanism behind how pore size affects chondrogenic differentiation.

Reference:

1. Hu J. Biomaterials. 2009;30(28):5061-5067.