Microribbon-based hydrogels guided mesenchymal stem cells to undergo endochondral ossification *in vivo*

Bogdan Conrad[1], Xinming Tong[2], Fan Yang[2, 3], Ph.D.
[1] Program of Stem Cell Biology and Regenerative Medicine, [2]Orthopaedic Surgery, [3]Bioengineering,
Stanford University, Stanford, CA

Statement of Purpose: Hydrogels are popular biomaterials for tissue engineering; however, most hydrogels lack macroporosity that is critical for bone-healing activities including cell and blood-vessel ingrowth and new tissue deposition. Furthermore, most hydrogels also lack the mechanical strength for engineering load-bearing tissues such as bones. We have recently reported development of gelatin-based, microribbon (µRB)-like elastomers that can photocrosslink into macroporous and highly flexible scaffolds. The goal of this study is to examine the efficacy of novel µRB-based scaffolds for supporting endochondral ossification of human mesenchymal stem cells (hMSCs) long term *in vivo* using a mouse subcutaneous model.

Methods: Gelatin-based µRBs were synthesized as we previously reported using wet-spinning. µRBs were prefixed by glutaraldehyde after being coated with methacrylate anhydride to enable photocrosslinking among µRBs to form macroporous scaffolds. hMSCs were encapsulated in gelatin µRB-based scaffolds or conventional gelatin hydrogels (HG) at 15 M/mL. Acellular µRB or HG scaffolds were also included as controls. To induce endochondral ossification, all samples were first cultured in chondrogenic medium for 14 days *in vitro*, then subsequently transplanted subcutaneously into nude mice for an additional 3 or 9 weeks. Outcomes were analyzed using mechanical testing and histology.

Results: The gross morphology revealed that hMSCs encapsulated in µRBs completely remodeled the scaffold while cell-free scaffolds degraded entirely after 9 weeks *in vivo*. The HG control group also showed partial remodeling, although it was limited to the core of the scaffold. Biochemical assays and mechanical testing demonstrated that the µRB scaffold supported the deposition of extracellular matrix (ECM) components, which resulted in a 21-fold increase in compressive moduli in cell-containing constructs after 3 weeks *in vivo* and a 325-fold increase after 9 weeks *in vivo* compared to day 1 measurements. Histology demonstrated that macroporosity of the µRB-based scaffold supported homogeneous ECM deposition throughout the construct as visualized by Safranin-O, Alizarin Red S, Alkaline Phosphatase, Osteocalcin, and Collagen II staining (Figure 1). The successful endochondral ossification occurred *in vivo* between weeks 3 and 9.

Conclusions: Our results demonstrated that µRB-based scaffolds enhanced and accelerated endochondral ossification of MSCs *in vivo* without additional growth factors. Coupled with the advantages of macroporosity and significant increase in mechanical property, we envision such µRB-based scaffolds will provide novel injectable macroporous scaffolds to enhance stem cell-based therapies for repairing long bone defects. The MSCs underwent endochondral ossification pathway without addition of any BMPs, which would substantially accelerate its clinical translation by reducing the cost and undesirable side effects.

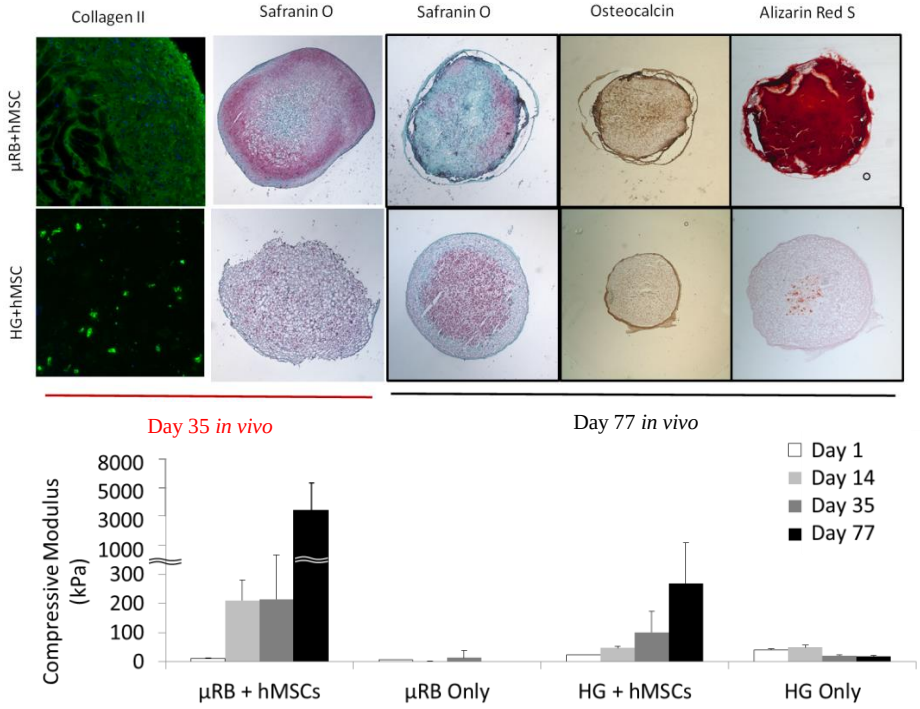

Figure 1. µRB-based scaffolds led to marked enhanced cartilage matrix formation 3 weeks after subcutaneous implantation. Long term *in vivo* results showed µRB scaffolds subsequently accelerated endochondral ossification with decreased GAG and enhanced mineralized bone formation. The matrix formation correlated with the compressive modulus showing a marked increase during the cartilage stage (Day 1 – Week 3, ~200kPa) and a dramatic increase after ossification (Week 9, ~3000kPa).