3D Bioprinted Bone-Mimetic Microenvironment for Investigating Primary Osteocyte Senescence

Maryam Tilton*, Megan M Weivoda, James L Kirkland, Lichun Lu
The University of Texas at Austin, Austin, TX, USA
Mayo Clinic, Rochester, MN, USA
Email: maryam.tilton@austin.utexas.edu

Disclosures: Maryam Tilton (N), Megan M Weivoda (N), James L Kirkland (N), Lichun Lu (N)

INTRODUCTION: Osteocytes, the most abundant bone cells, serve as strain gauges within the bone microenvironment, transducing the mechanical signals to biochemical signals to orchestrate bone-forming and bone-resorbing cells (i.e., osteoblasts and osteoclasts, respectively).[1] Despite their crucial role in bone remodeling, understanding osteocyte mechanobiology, especially in the context of aging, remains incomplete due to challenges in isolating and controlling mechanical stimuli in vivo.[2] Traditionally, investigations into osteocyte properties and functions have heavily relied on mice-derived cell lines such as MLO-A5, MLO-Y4, and IDG-SW3 after extensive *in vitro* manipulations under 2D experimental conditions. Notably, these cell lines fail to express the complete repertoire of markers associated with primary osteocytes, creating a significant knowledge gap in osteocyte mechanobiology. Our team has previously explored the impact of cellular senescence on age-related bone loss both in vivo and under static 2D culture conditions.[3-4] Findings from these studies underscored the prevalence of senescent cells, including osteocytes, in the aging bone microenvironment. Targeting these senescent cells demonstrated both anti-resorptive and anabolic effects on bone. However, our earlier investigations lacked detailed insights into the mechanisms by which the age-related accumulation of senescent cells influences osteocyte properties and functions. This study aims to bridge this gap by developing a novel 3D bioprinted bone-mimicking microenvironment for 3D co-culture of primary osteocytes, both healthy and senescent, isolated from female and male mice. Through this model, we seek to unravel the intricate changes in osteocyte mechanobiology associated with aging, providing crucial insights into the underlying mechanisms of age-related alterations in bone health.

METHODS: Primary osteocytes were isolated from the vertebrae of male and female C57BL/6 wild-type mice. A highly enriched osteocyte population was obtained from digestion cycles 7 to 9, followed by centrifugation and culture on type I-collagen-coated plates (Figure 1A). To facilitate visible light-induced 3D bioprinting, a photocrosslinkable ink was formulated using synthesized GelMA (10% w/v), PEGDA (10% w/v), and rat tail type I-collagen (0.15 mg/mL) (Figure 1A). Following two passages, a subset of cultures underwent exposure to 10 Gy cesium irradiation to induce in vitro senescence. Subsequently, visible light-induced 3D bioprinting was employed to create cell-laden scaffolds with controlled ratios of senescent to non-senescent osteocytes (ranging from 0 to 100% and 15 to 85%). We comprehensively evaluated the 3D composite hydrogel scaffolds, including assessments of swelling, degradation, hydrophilicity, and compressive mechanical properties. In addition to these evaluations, in vitro cytocompatibility assessments were performed, and expression analyses of key osteocyte and senescence-associated markers were conducted.

RESULTS: Key findings from this study are summarized in Figure 1. The visible light-induced 3D bioprinting technique exhibited robust cell viability. F-actin and nuclei immunofluorescence images highlighted the presence of healthy osteocytes on 3D printed scaffolds for both female and male cells. RNA expression analysis revealed elevated expression of key osteocyte markers (e.g., Sost, Dmp1) in 3D cultures compared to 2D, with distinct sex-associated differences. Notably, the normalized senescence-related markers (e.g., p21, p16, MMP12, IL6) displayed increased expression patterns in the 3D co-cultures.

DISCUSSION: Elevated expression of osteocyte markers in 3D cultures suggests a closer mimicry of in vivo conditions. Sex-associated differences in marker expression underscore the interplay between sex and the 3D culture environment. The distinct, normalized increasing expression pattern of p21 hints at the complex effects of senescent cells, inducing senescence in healthy osteocytes within the 3D co-culture microenvironment. In summary, these findings shed light on the potential of the 3D bioprinted microenvironment for understanding primary osteocyte behavior and gene expression, in the context of aging.

SIGNIFICANCE/CLINICAL RELEVANCE: The aging population is growing rapidly with over 11,000 Americans turning 50 daily, and by 2030, all boomers will be at least aged 65. Aging itself is considered the greatest risk factor for most of the world's chronic diseases, including bone loss. The significant socio-economic impact of age-associated fragility fractures makes mechanistic understanding of mechano-biological cues underlying the age-related bone loss of great importance. The long-term goal of this work is to determine the local and systemic role of cellular senescence in osteocyte mechanobiology and, ultimately, advancing the development of therapies for bone loss.

ACKNOWLEDGEMENTS: During the early stages of this project, MT was supported by National Institute of Health grant (T32AR56950) for Musculoskeletal Research Training at Mayo Clinic, prior to her transition to UT Austin.

RFERENCES: 1. Wright NC, Looker AC, Saag KG, et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Miner Res. 2014;29(11):2520-2526.
2. Hemmatian H, Bakker AD, Klein-Nulend J, van Lenthe GH. Aging, Osteocytes, and Mechanotransduction. Curr Osteoporos Rep. 2017;15(5):401-411.
3. Khosla S, Farr JN, Tchkonia T, Kirkland JL. The role of cellular senescence in ageing and endocrine disease. Nat Rev Endocrinol. 2020;16:263-275.
4. Farr JN, Xu M, Weivoda MM, et al. Targeting cellular senescence prevents age-related bone loss in mice. Nat Med 2017 239. 2017;23(9):1072-1079.

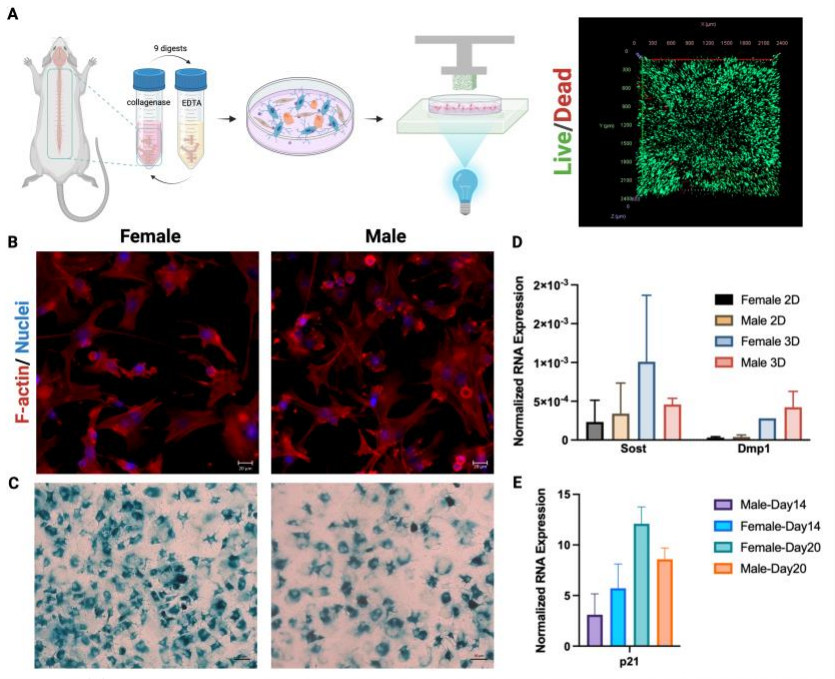

Figure 1: (A) Schematic depiction of primary cell isolation and visible light-induced 3D bioprinting showcasing improved viability. (B) Three-day culture of primary osteocytes on 3D printed composite hydrogel scaffolds. (C) Induction of senescence through irradiation. (D) Expression patterns of key osteocyte markers, 2D vs. 3D cultures. (E) Increased expression of senescence markers.