Title: Investigating the Role of S53P4 Bioactive Glass in Osteogenic Differentiation of 3D Bioprinted Human Bone Marrow Stem/Stromal Cells with Animal-Free Bioinks

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Abstract
There is a growing demand in biomedicine for tissue models that closely replicate native tissues for studying tissue development, disease mechanisms, and therapeutic innovations. Bone tissue models are essential for investigating conditions such as bone defects and fractures, osteoporosis, and cancer metastases to bone. Bioactive glass (BAG), known for its biocompatibility and bone-stimulating properties, has shown promise in orthopedic and dental applications. Nanofibrillated cellulose (NFC), a biodegradable and biocompatible material derived from wood, has demonstrated potential in improving bioinks for tissue engineering.

This study investigated the effects of S53P4 BAG extract on 3D bioprinted human bone marrow stem/stromal cells using two animal-free bioinks containing modified hyaluronic acid, collagen type I, and either unmodified hyaluronic acid or NFC. The 3D bioprinted constructs were cultured in osteogenic medium with or without BAG extract. Cell viability, functions, proliferation, and osteogenic differentiation were assessed using Live/Dead assays, immunocytochemistry, CyQuant assay, quantitative reverse transcription polymerase chain reaction (RT-qPCR), and ALP activity assays. Mineralization and mechanical properties were evaluated via micro-computed tomography (μCT), and compression testing.

Results showed that cells remained viable and exhibited elongated morphology, indicating good cell attachment. The cells expressed osteogenic markers like osteocalcin and collagen I, and showed varying ALP activity. BAG extract enhanced cells' elongated morphology and osteocalcin expression. Both BAG and NFC improved mineral deposition and the mechanical strength of the constructs.

These findings support the use of BAG and NFC in bioinks for bone tissue engineering and highlight the need for further research into their specific roles and mechanisms.