



Bioengineered Silk Scaffolds and Nanoparticles for Targeted miRNA Delivery in Bone Tissue Engineering

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Objective

To evaluate the potential of silk-based 3D-printed scaffolds and silk nanoparticles (SNPs) for microRNA (miRNA) delivery in the context of alveolar bone regeneration.

- Alveolar bone loss arises from tooth loss, periodontal disease, trauma, or pathology and leads to defects which complicating implant placement.
- Guided bone regeneration (GBR) is a well known procedure for augmenting the defects but complications including membrane exposure, closure challenges, and need for secondary surgery are commonly found.
- 3D printing technology enables scaffold-based alternatives for bone reconstruction.
- Silk was explored as miRNA delivery approach via 3D-printed scaffolds for bone regeneration.

Methods

- Silk fibroin (SF) extracted from cocoon was blended with Hydroxyapatite (HA) and Tricalcium phosphate(TCP) for printable bone cement (Fig.1).
- Optimal mix identified by mechanical testing and used to create 3D-printed scaffolds. SEM was used to analyze its morphology.
- Human mesenchymal stem cells (hMSCs) were seeded on the scaffold and SEM was used to evaluate its adhesion and proliferation.
- SNPs synthesized from SF solution were loaded with miRNAs (Fig.2)
- Transfection efficiency compared with commercial Lipofectamine tested by confocal microscopy, Alamar Blue, qRT-PCR.

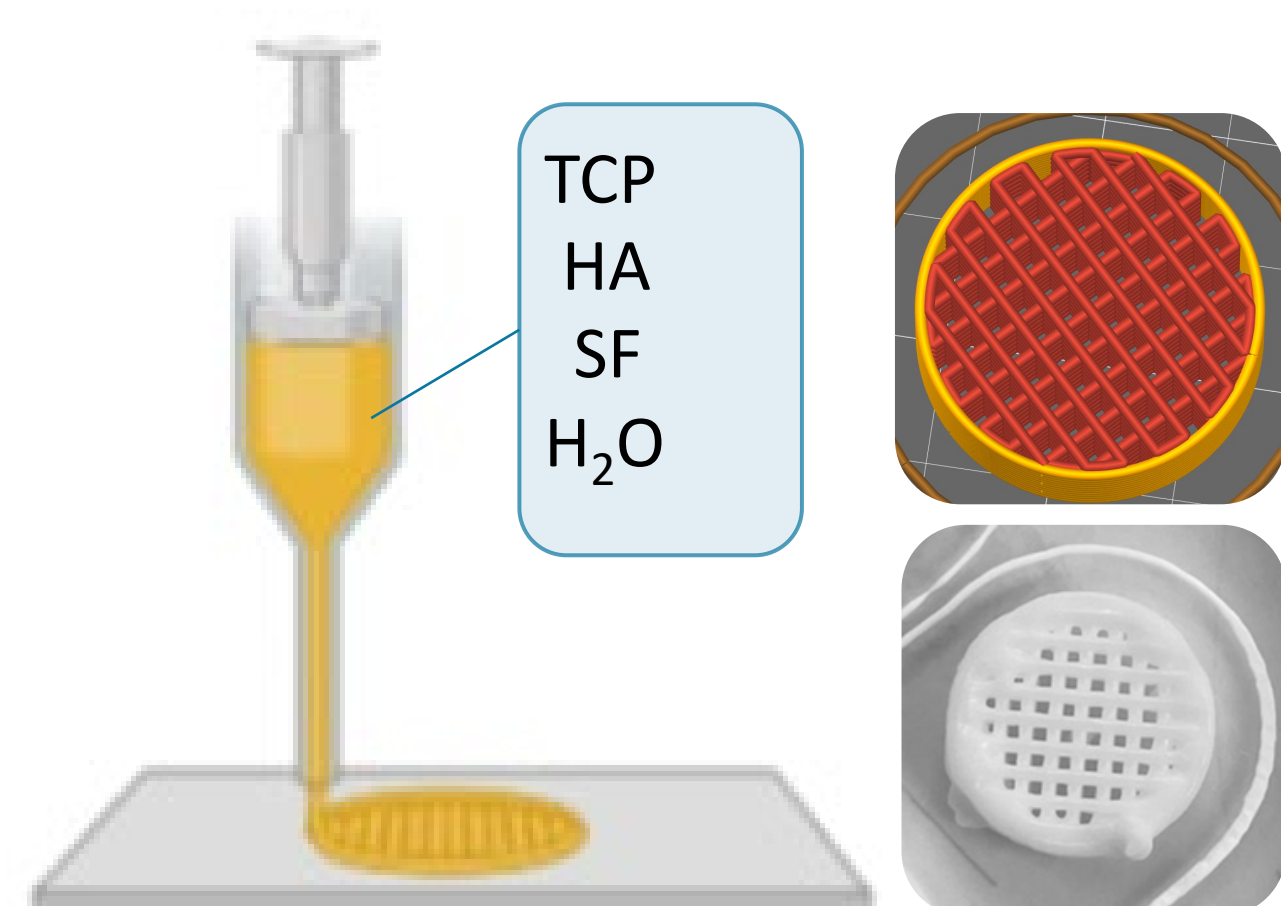


Figure 1. A mixture of β -tricalcium phosphate (TCP), hydroxyapatite (HA), silk fibroin (SF), and water was formulated into a paste-like material and processed using extrusion-based 3D printing to fabricate scaffolds.

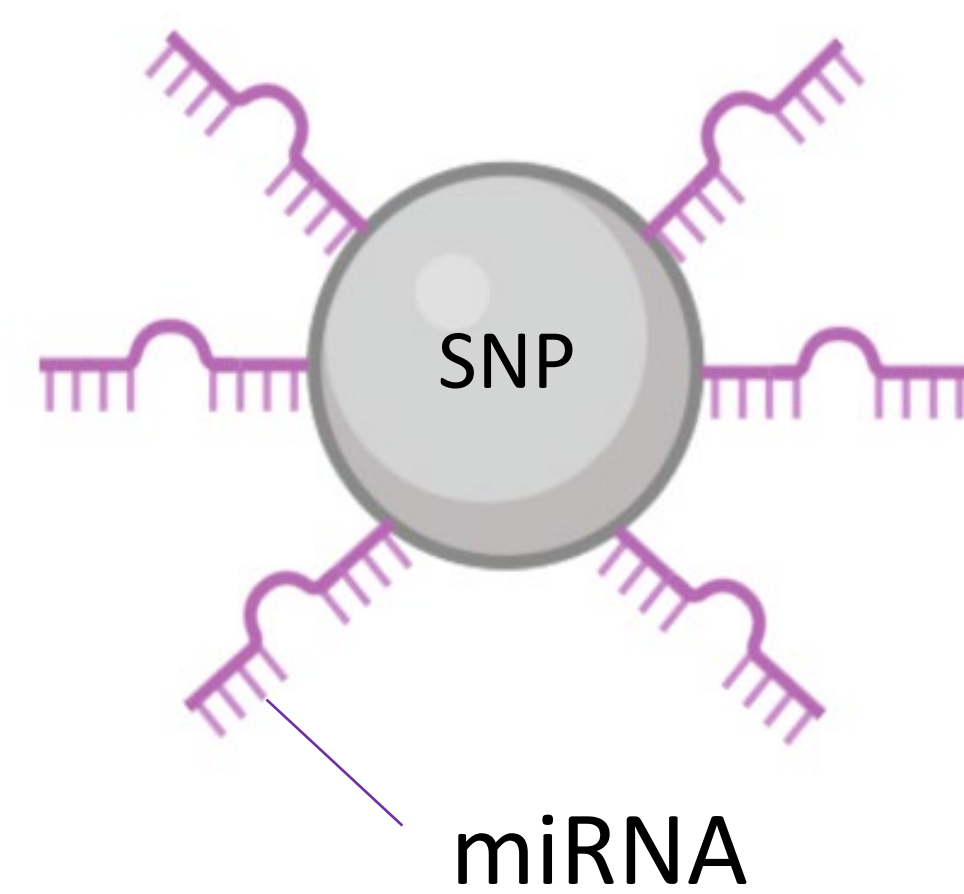


Figure 2. Silk nanoparticles (SNPs) were loaded with miRNA to serve as carriers for targeted delivery, enabling modulation of cell characteristics and functions.

Results

- 50:50 HA: β -TCP ratio of bone cement could create scaffold with uniform filaments, optimal pore structure, and highest compressive strength (Figs.3,4,6)
- The scaffold can support hMSC viability and proliferation (Fig.5)
- SNPs successfully transfected cells (Fig.7)
- SNPs showed lower cytotoxicity with higher metabolic activity (Fig.8) but lower miRNA transfection efficiency than Lipofectamine (Fig.9)

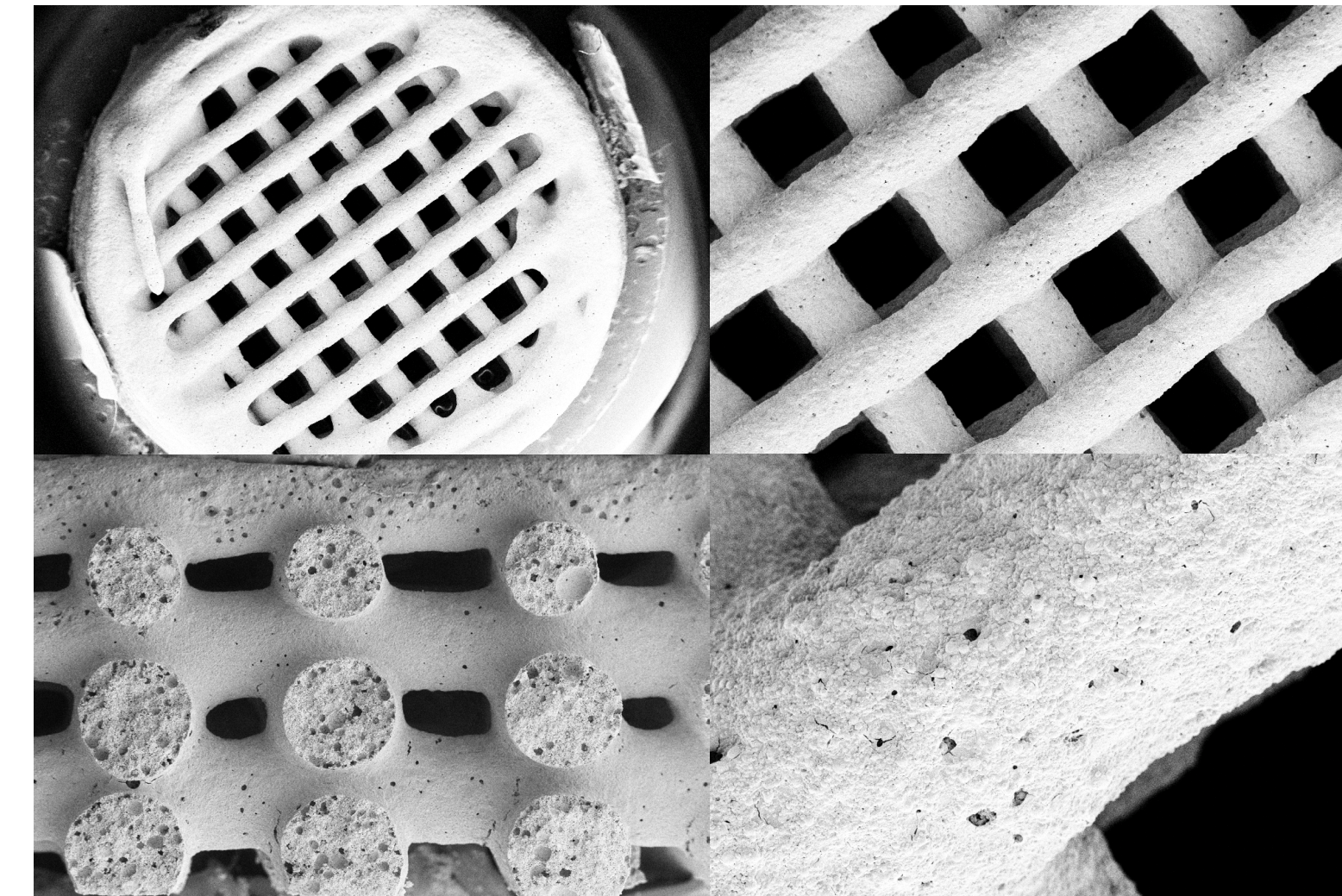


Figure 3. SEM images of the 3D-printed scaffold show a well-defined architecture with appropriate pore size and filament dimensions. Intrafilament porosity is also evident.

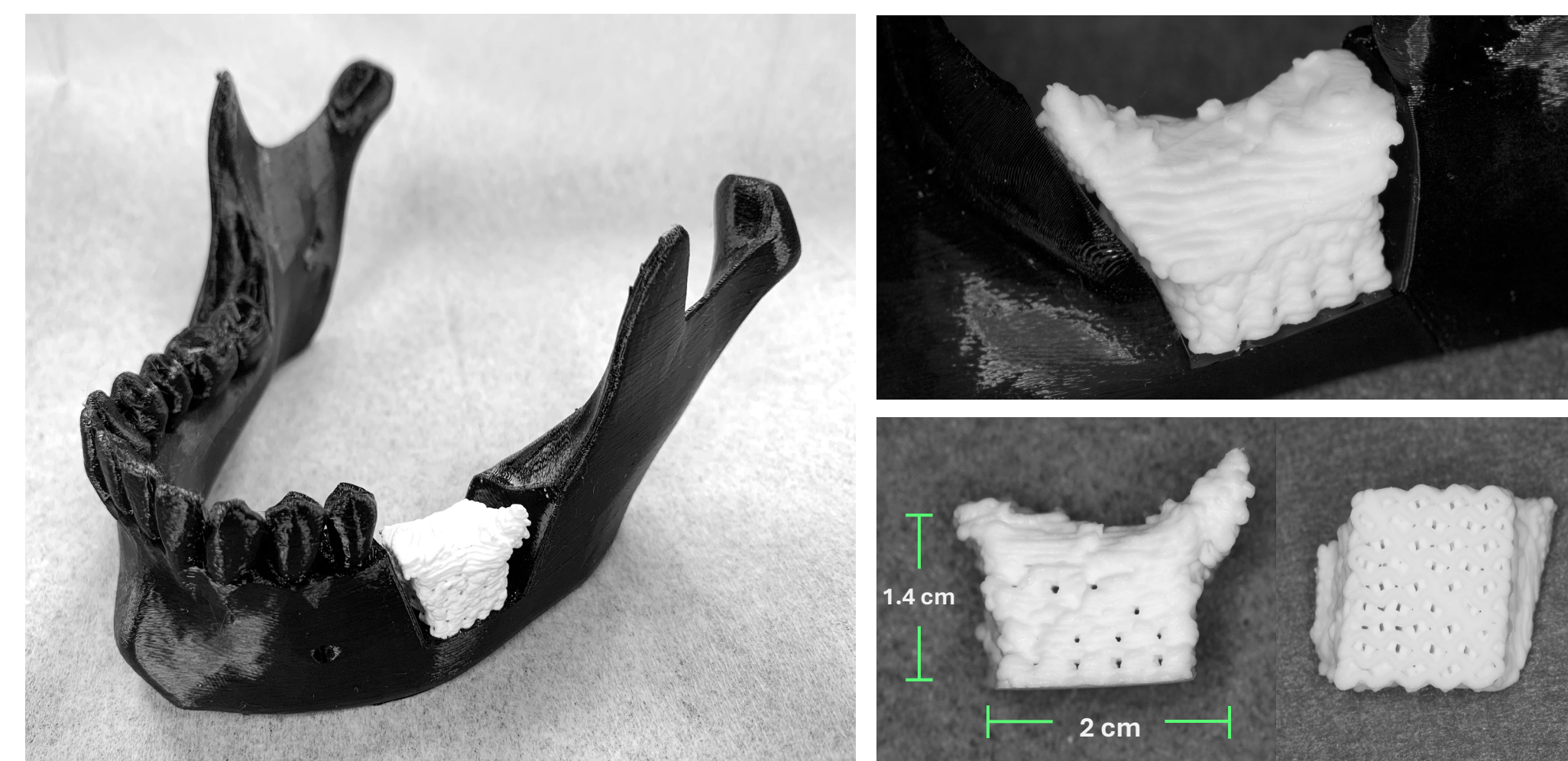


Figure 4. Simulation of a 3D-printed scaffold applied to an alveolar bone defect demonstrates its potential to augment deficient bone height and volume (Courtesy of Dr. Ayman Banjar).

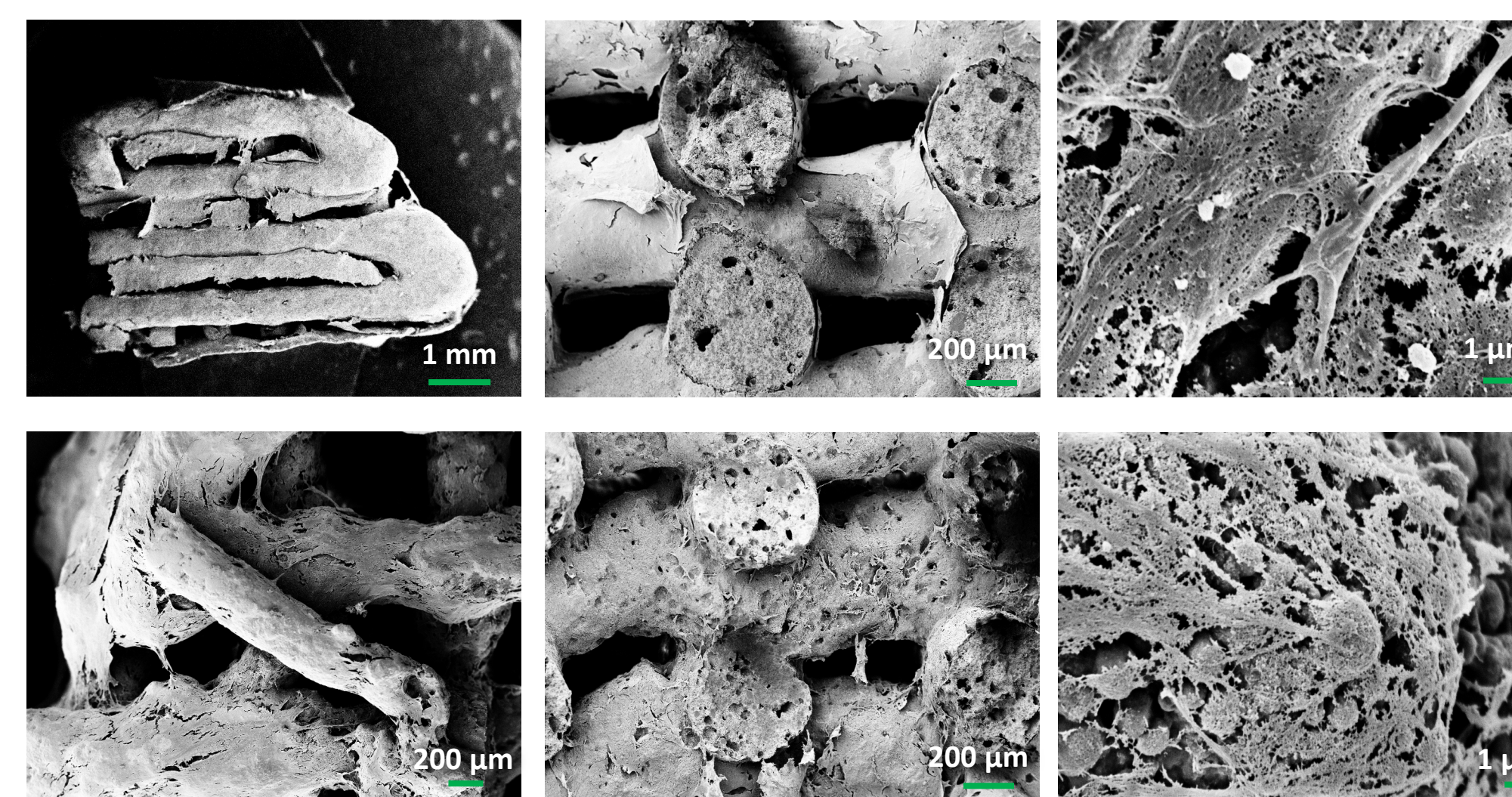


Figure 5. SEM images of scaffolds cultured with hMSCs for 35 days demonstrate support for cell adhesion and proliferation (Courtesy of Dr. Ayman Banjar).

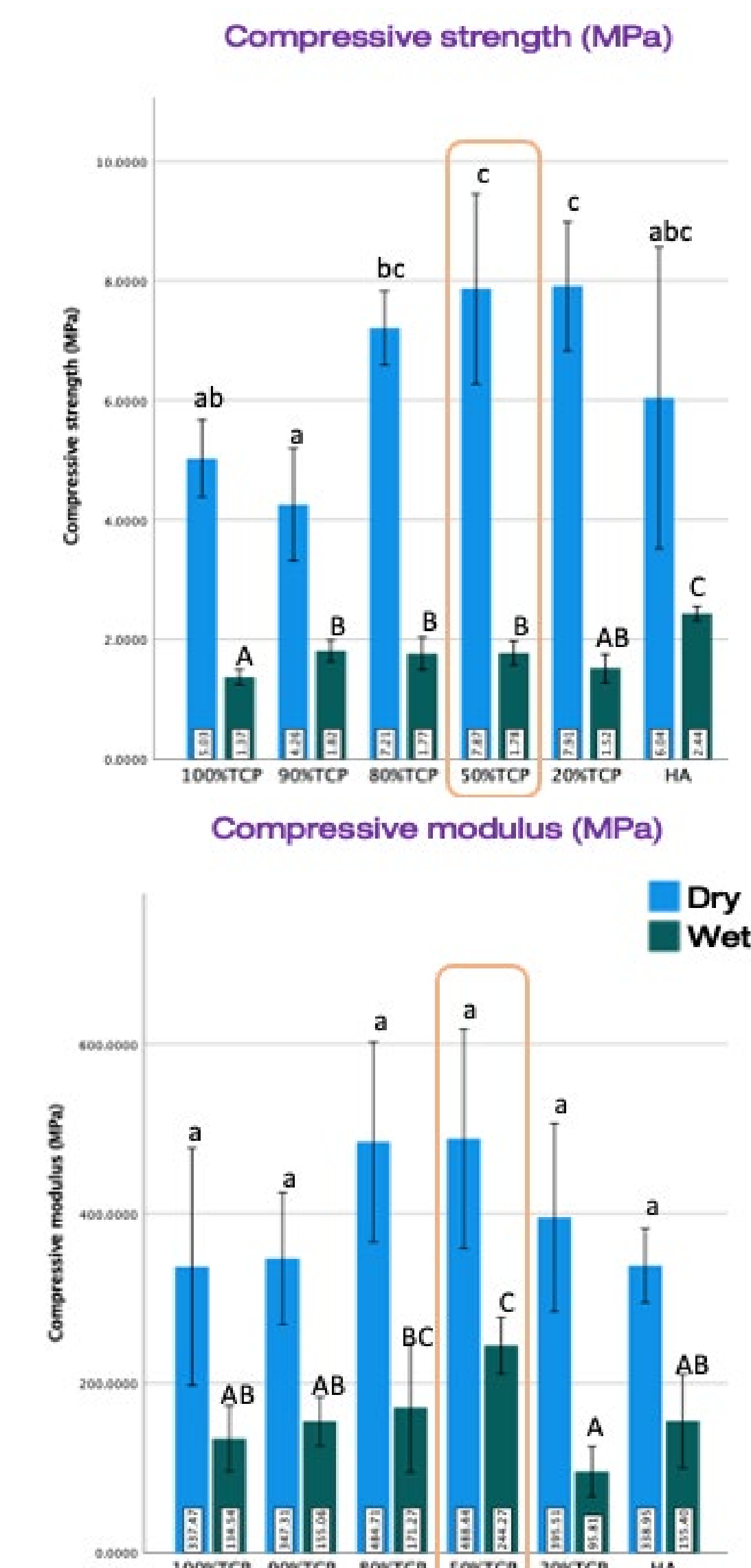


Figure 6. Mechanical properties of different bone cement formulations, showing compressive strength and modulus under both dry and wet conditions. The 50:50 TCP:HA ratio exhibited the highest values and was selected for further experiments.

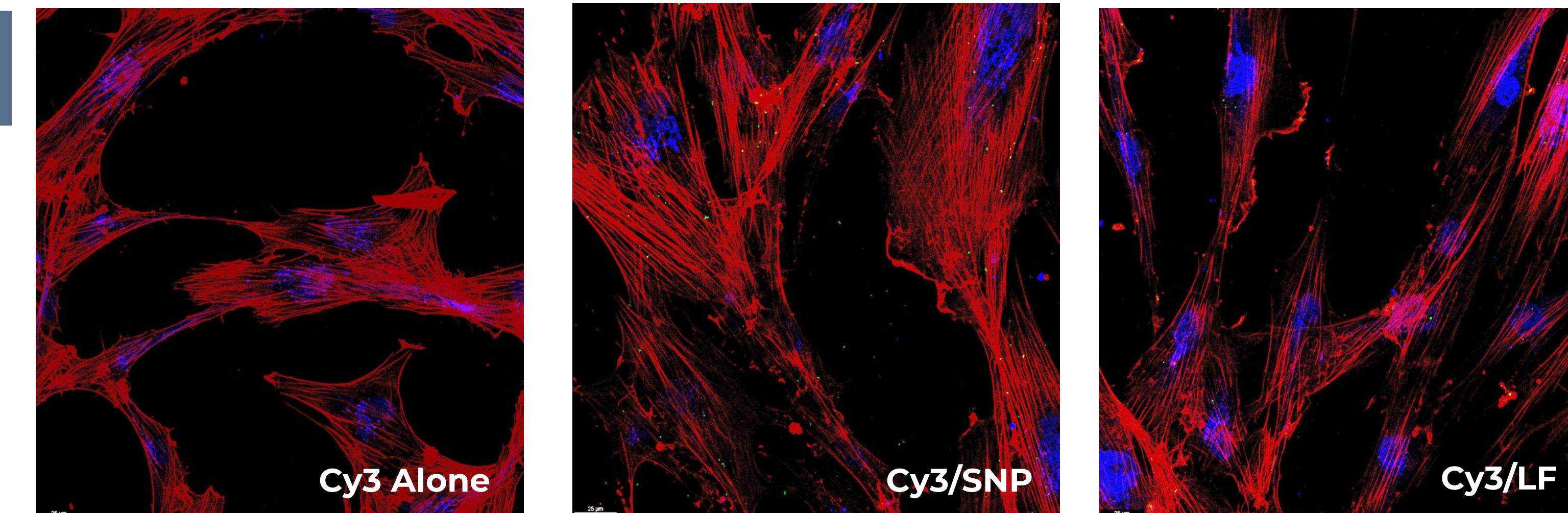


Figure 7. Confocal microscopy images show that silk nanoparticles (SNPs) coupled with Cy3-labeled miRNA were successfully transfected into hMSCs, indicated by green fluorescence. Cy3 signal was observed in cells transfected with Cy3/SNP and Cy3/LF, but not in the control without nanoparticles.

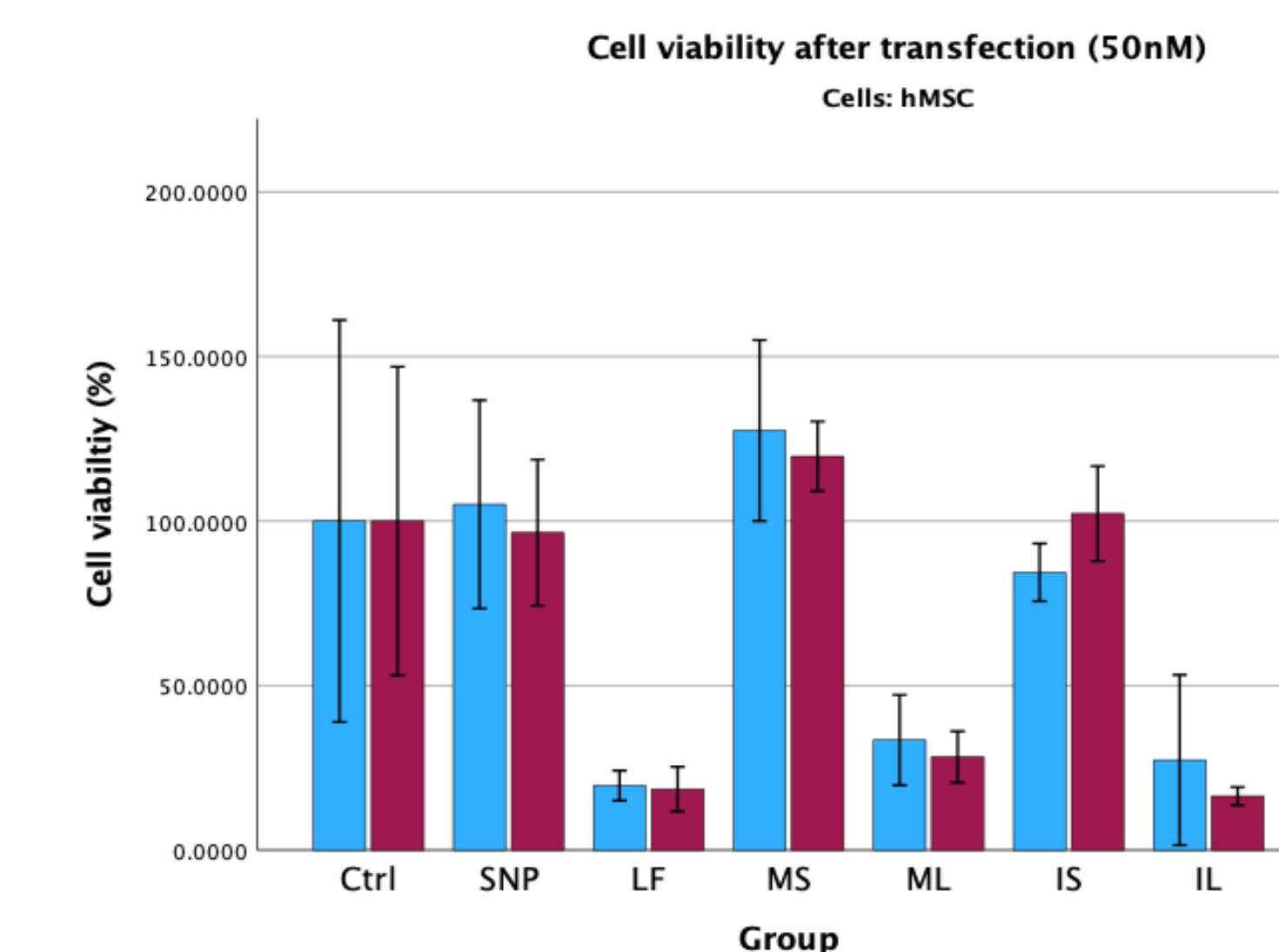


Figure 8. Alamar Blue assay results show that cells transfected with SNPs (SNP, MS, and IS groups) maintained comparable viability to the control, while Lipofectamine resulted in significantly reduced cell viability after transfection.

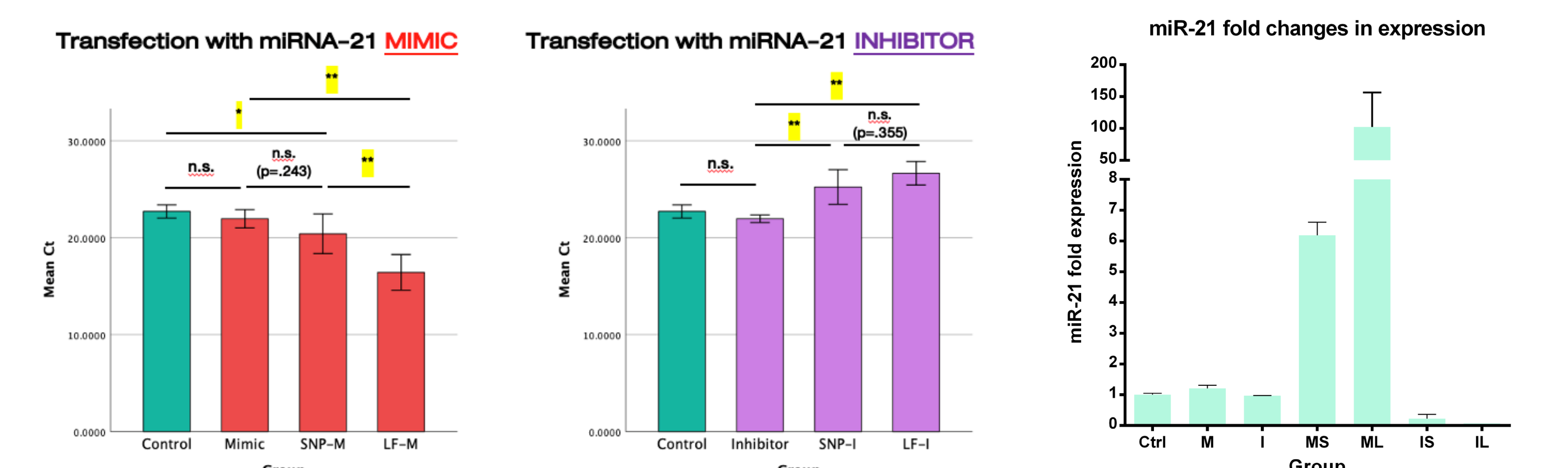


Figure 9. miR-21 expression levels in hMSCs changed after transfection with miRNA/SNP and miRNA/LF complexes. Both SNP and LF could modulated intracellular miRNA levels.

Conclusion and Future works

- 3D-printed silk/HA/ β -TCP scaffolds provide biocompatible architecture to support bone regeneration.
- Silk nanoparticles offer safe miRNA delivery.
- Further studies are required to validate efficacy for its bone regeneration capacity especially in vertical bone deficiency.