



3D-Printed Allograft/Alginate/Gelatin Scaffolds Coated with Platelet-Rich Fibrin or Adipose Stromal Vascular Fraction for In Vitro Osteogenesis

Sahar Baniameri ¹, DDS, PgC, Arash Khojasteh ² DDS, OMFS, PhD

¹ Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA

² Department of Cranio-Maxillofacial Surgery, University Hospital, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium



Background:

Bone defects pose significant challenges to restoring functionality and preventing complications in implant therapy, necessitating innovative solutions to enhance bone regeneration. Tissue engineering has emerged as a promising strategy, integrating scaffolds, cells, and signaling molecules to facilitate osteogenesis. Among the patient-derived products, platelet-rich fibrin (PRF) and stromal vascular fraction (SVF) have shown immense potential due to their rich content of growth factors and stem cells. PRF, derived from blood, and SVF, obtained from adipose tissue, are easily accessible and clinically feasible, enhancing their practicality in regenerative medicine. PRF and SVF synergistically promote osteoblast differentiation and bone formation. In parallel, advancements in 3D printing have enabled the development of customizable scaffolds using materials like alginate, gelatin, and allografts. These scaffolds mimic the extracellular matrix (ECM) and support cell adhesion, growth, and differentiation, making them ideal for bone tissue engineering.

Aim:

To assess the osteogenic potential of 3D-printed allograft-alginate-gelatin scaffolds and evaluate the effects of PRF and SVF coatings on scaffold viability and osteogenic induction. Three study groups were analyzed:

1. Scaffold + SVF + Cells
2. Scaffold + PRF + Cells
3. Control (Scaffold + Cells)

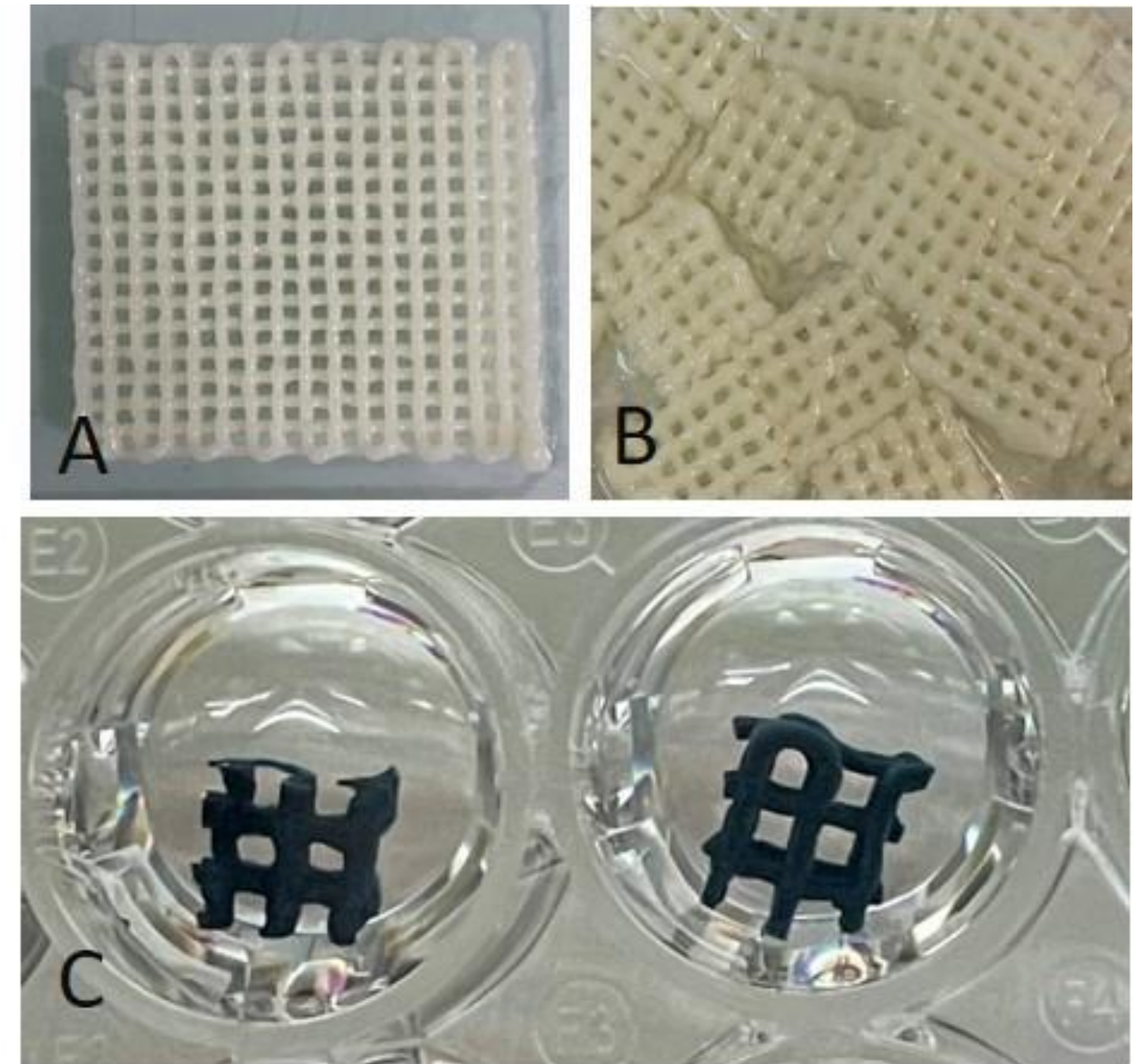


Figure 1. A) 3D printed plain allograft/alginate/gelatin scaffold, (B) Immersed scaffolds in genipin, and (C) Cross-linked scaffolds cut into desirable pieces.



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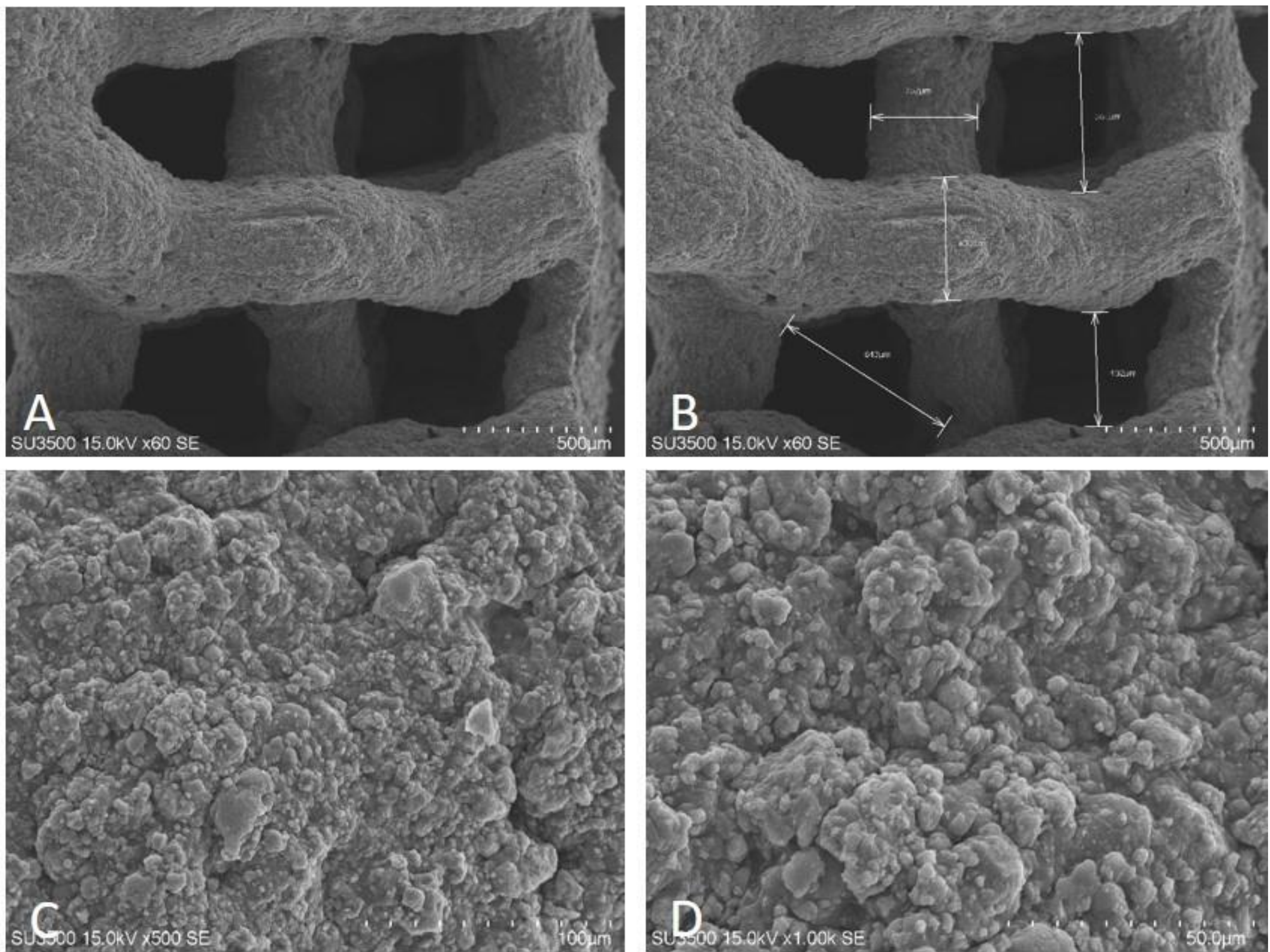


Figure 2. (A, B) SEM images of the strut (mean: 397 μ m), pore size (mean: 481 μ m), measurements, and (C, D) surface roughness of the allograft/alginate/gelatin scaffold causing cell adhesion.

Methods:

3D-printed gelatin, allograft, and alginate scaffolds were utilized, along with stem cells derived from the buccal fat pad and human-derived components (PRF, SVF). Cells were seeded onto scaffolds, both with and without SVF/PRF, and subjected to comprehensive assessments including adhesion, proliferation, differentiation (gene expression and protein secretion levels), penetration, and gene expression analysis over 14 days. The data was reported as mean $\pm$ standard deviation (SD). Two-way or one-way analysis of variance (ANOVA) was performed, followed by a Tukey post hoc test for multiple comparisons. Statistical significance was determined as a P-value below 0.05.

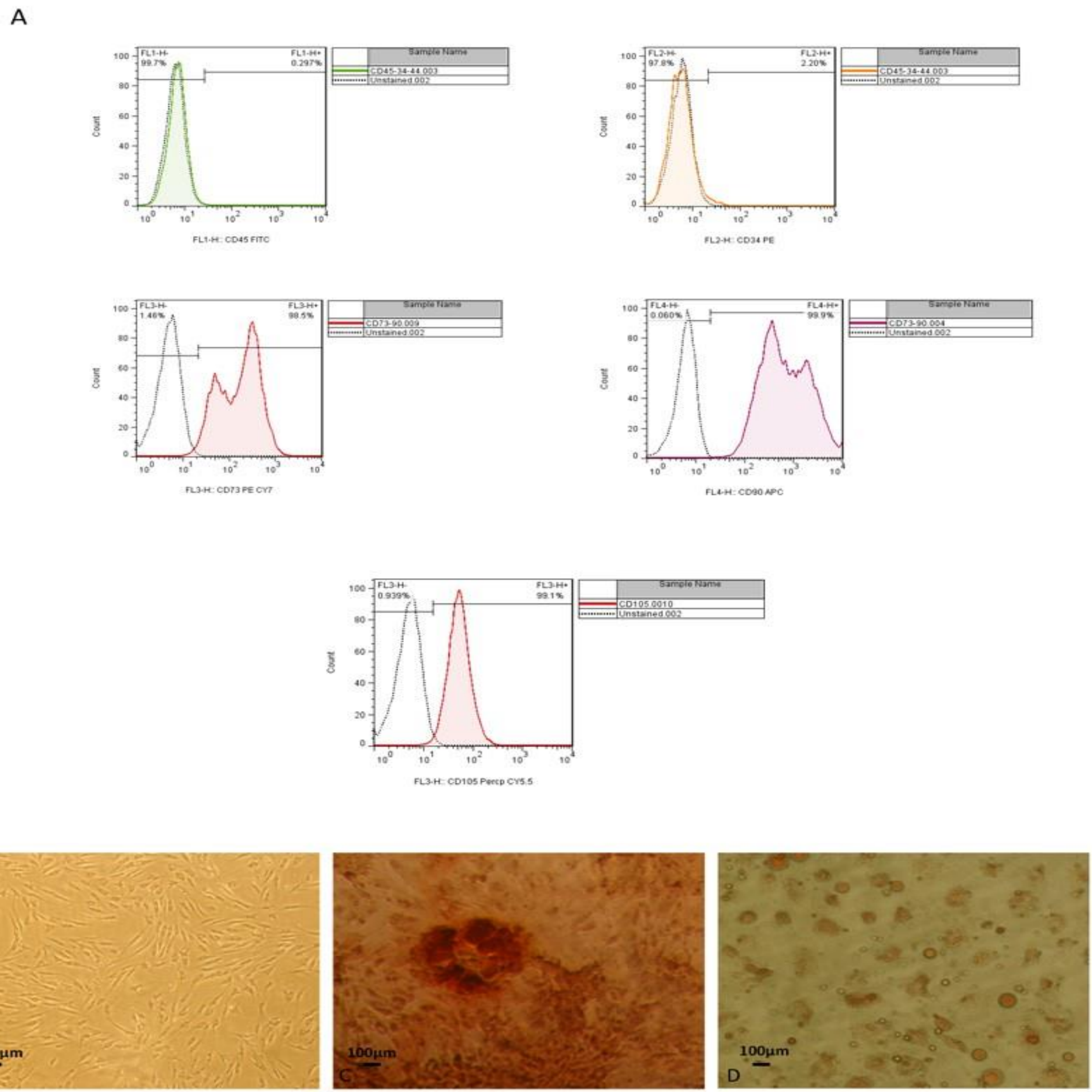


Figure 3. (A) Flow cytometric characterization of human BFPdSCs. (B) BFPdSCs after 4 days in growth medium. (C) Alizarin Red staining of MSCs in 2D culture 14 days after cell culturing in osteogenic medium. (D) Oil Red O staining of MSCs in 2D culture 21 days after cell culturing in adipogenic medium.



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Results:

Cell adhesion was assessed via SEM and DAPI staining after 3 days, showing enhanced adhesion in coated groups, with the PRF group performing better. Cell proliferation over 5 days, measured by MTT and Alamar Blue assays, showed an increase in cell numbers over time with significantly higher proliferation in the PRF group by day 5. Osteogenic differentiation over 14 days revealed increased ALP activity in both coated groups compared to the control with the PRF group exhibiting the highest activity at day 14. RT-PCR showed upregulation of osteogenic markers (BMP-2, OPN, RUNX2) and the angiogenic marker VEGF in coated groups. BMP-2 and VEGF peaked at day 7, while OPN increased from day 3 to day 7, and RUNX2 was notably higher in the PRF group at day 3 and day 7. Immunocytochemistry confirmed higher OCN and OPN expression in coated groups with the PRF group showing the highest levels. Giemsa staining revealed granular purple aggregates, indicating bone matrix formation predominantly in PRF-coated scaffolds.

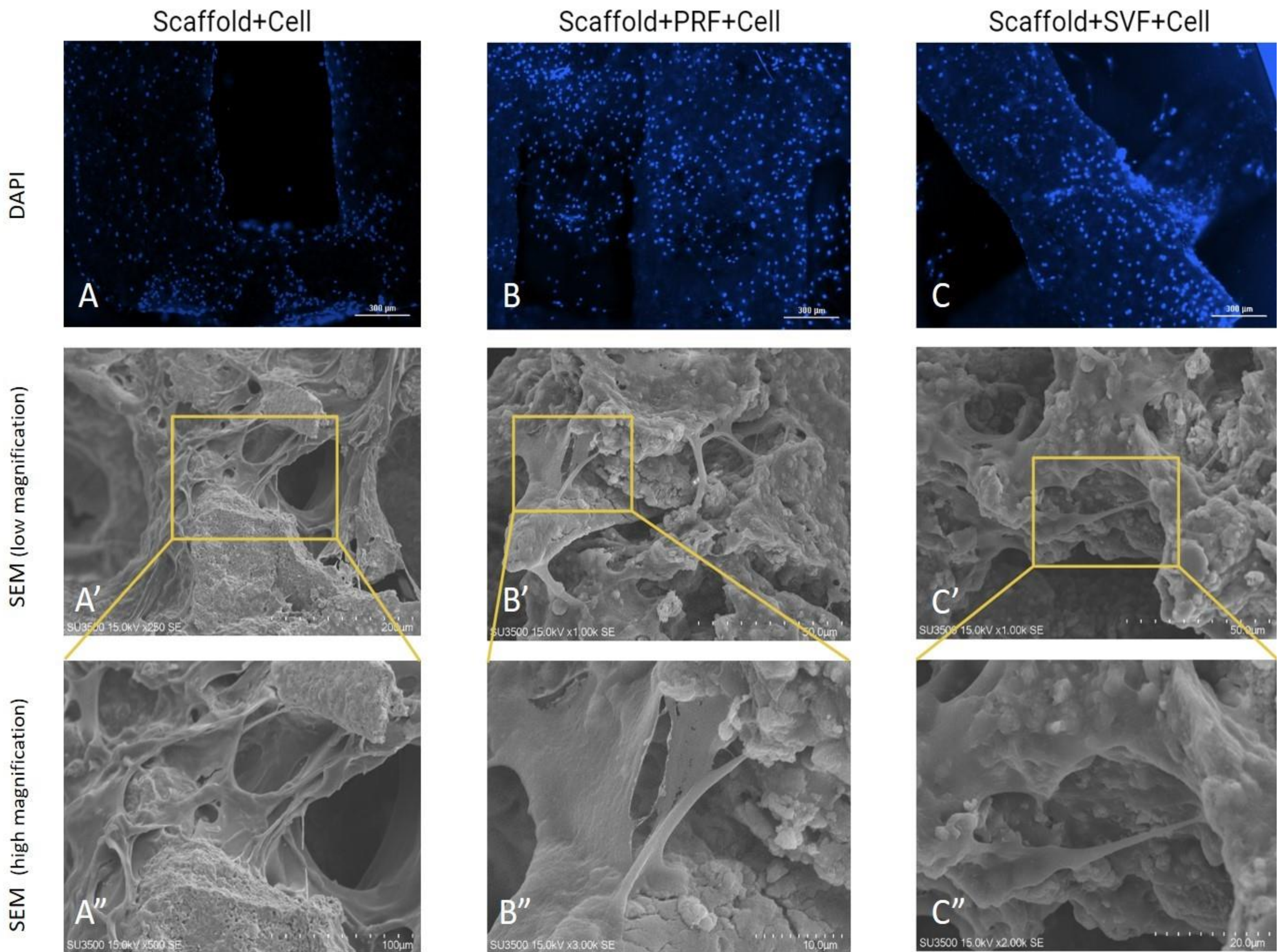


Figure 4. DAPI staining on day 3 showed higher cell attachment in Scaffold+PRF+Cell and Scaffold+SVF+Cell groups vs. control. SEM images at low (A', B', C') and high (A'', B'', C'') magnifications confirmed these findings.

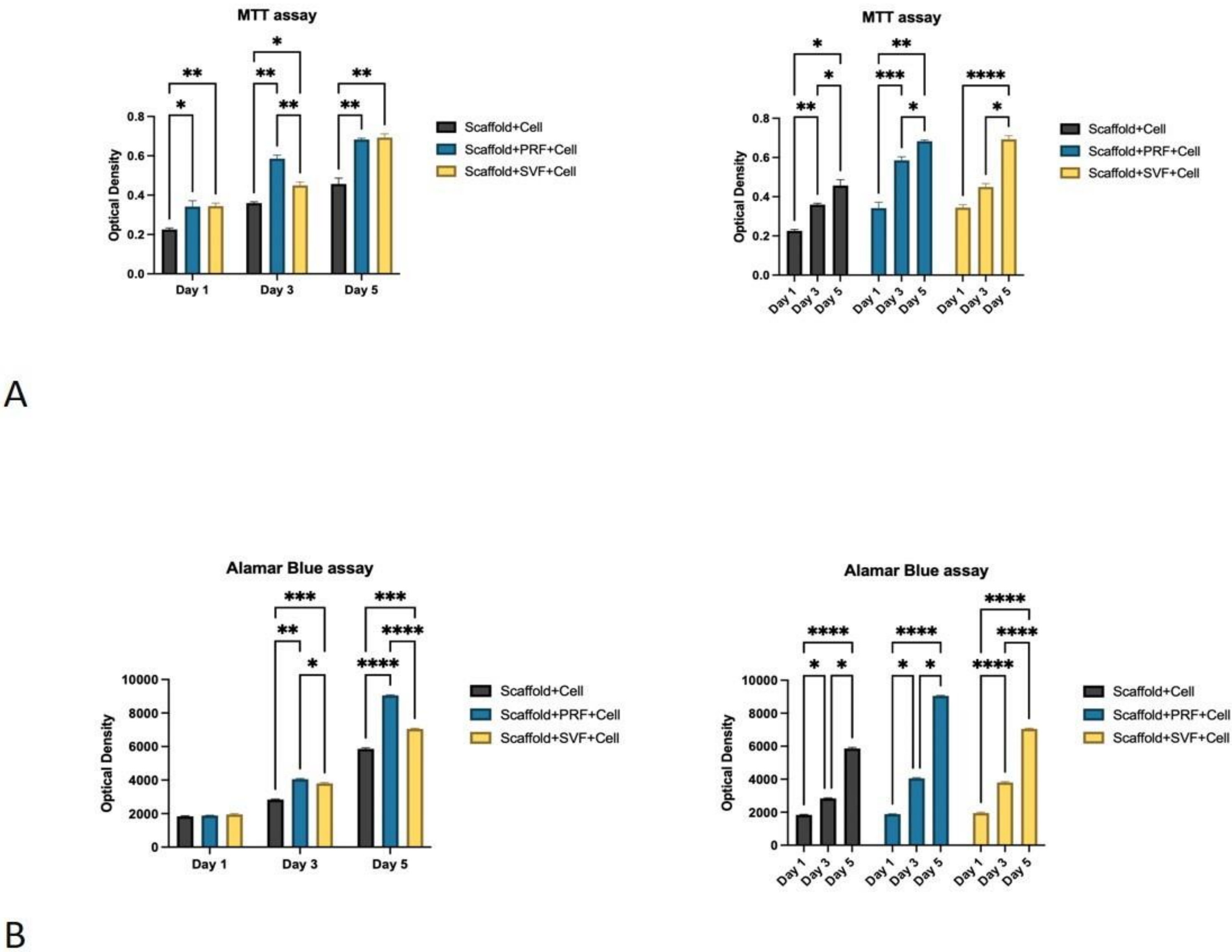


Figure 5. MTT and Alamar Blue assays showed increased growth, with higher proliferation in Scaffold+SVF+Cell and Scaffold+PRF+Cell (*P < 0.05—****P < 0.0001).



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Conclusion:

This study demonstrated that incorporating SVF and PRF into alginate/gelatin/allograft scaffolds created a favorable environment for mesenchymal stem cell proliferation and osteogenic maturation. Both SVF and PRF significantly enhanced stem cell differentiation, with the Scaffold+PRF+Cell group showing superior adhesion and gene/protein expression compared to other groups. These findings highlight the potential of this scaffold system as a promising alternative for tissue-engineered bone grafts, though further optimization and evaluation are required.

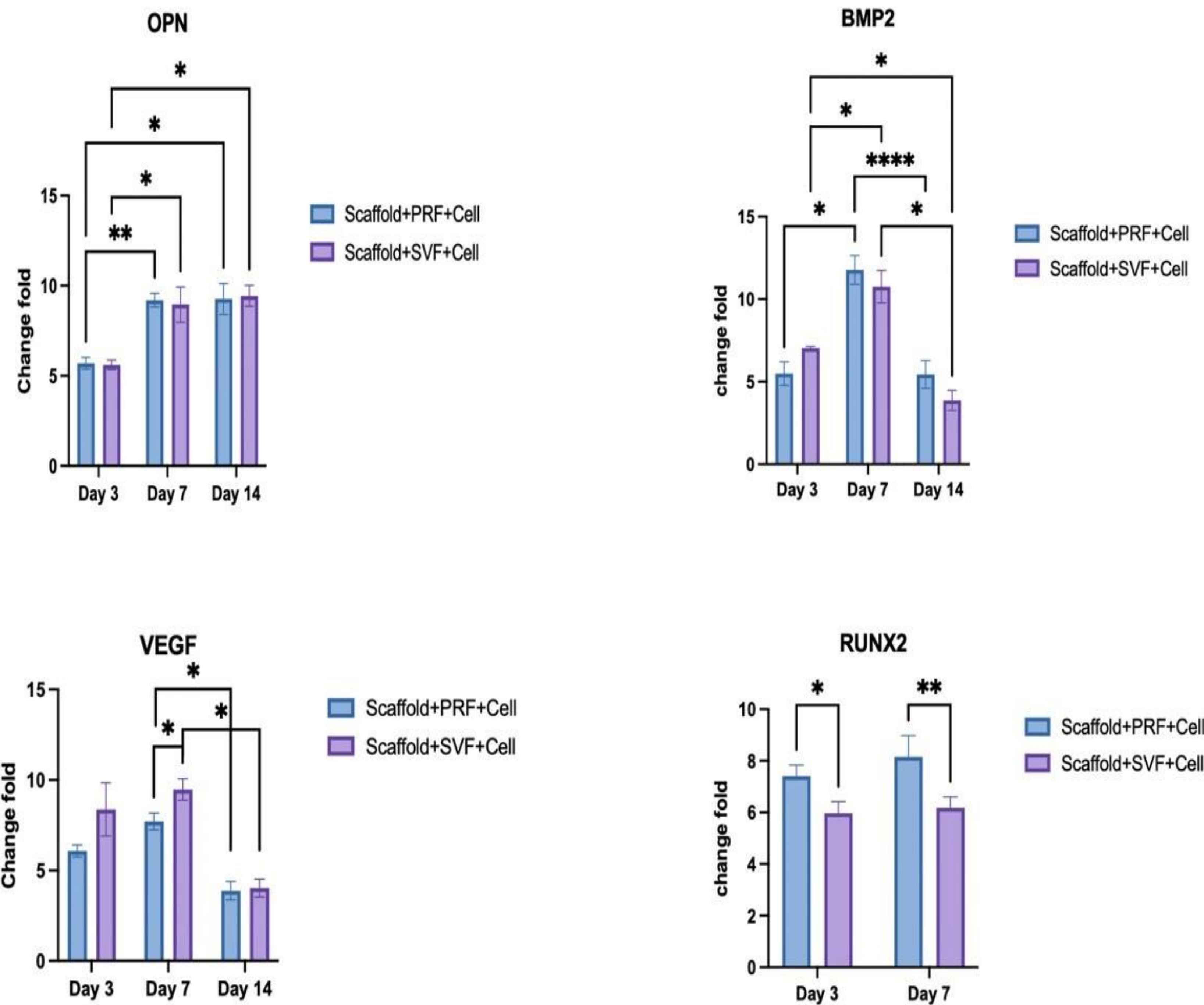


Figure 6. Scaffold+PRF+Cell and Scaffold+SVF+Cell showed elevated RUNX2, VEGF, BMP2, and OPN expression vs. control. Significant differences were observed between groups (*P < 0.05—****P < 0.0001).

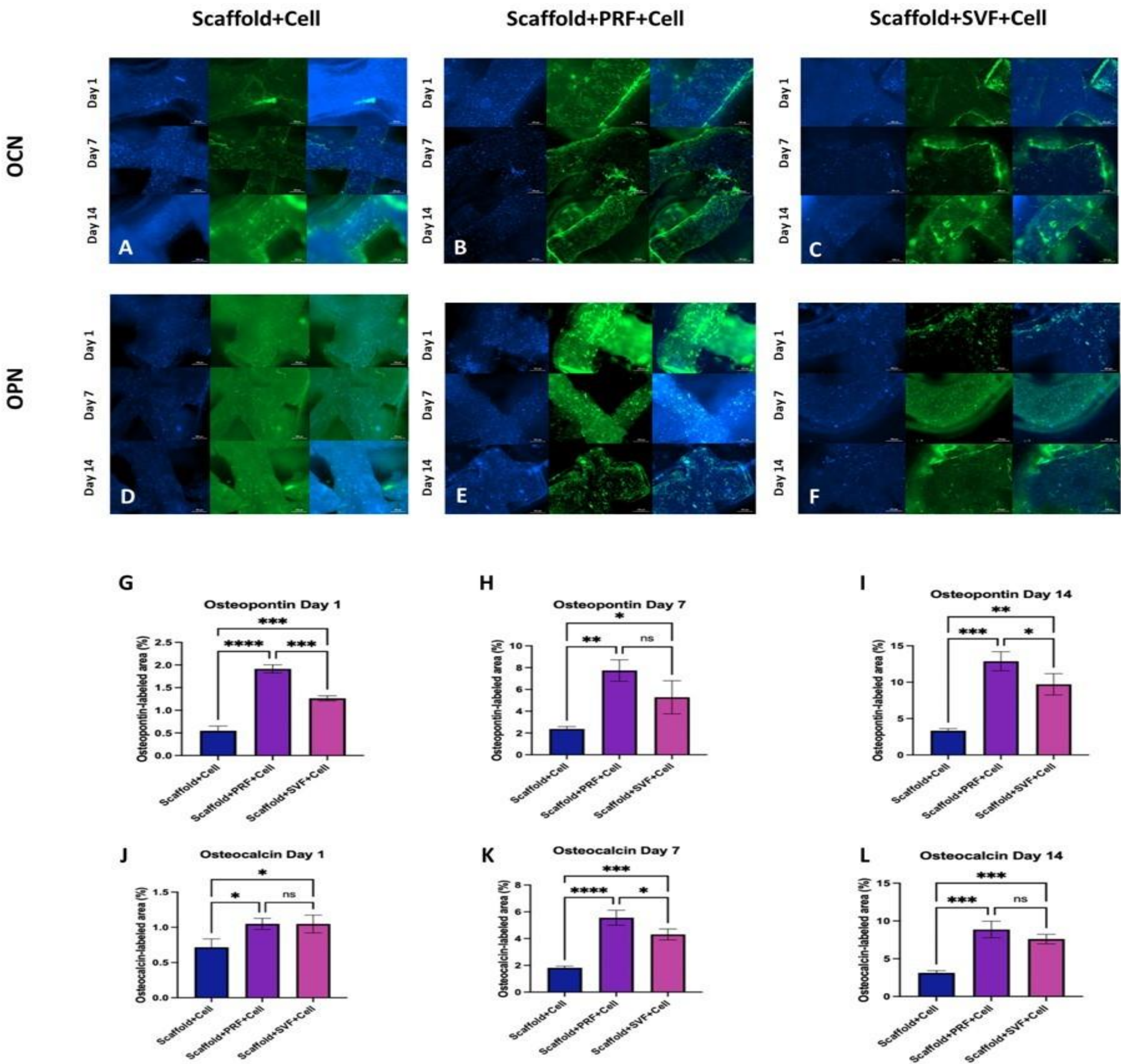


Figure 7. Scaffold+PRF+Cell and Scaffold+SVF+Cell showed higher OCN and OPN expression vs. control across days, with Scaffold+PRF+Cell often outperforming (*P < 0.05—****P < 0.0001).



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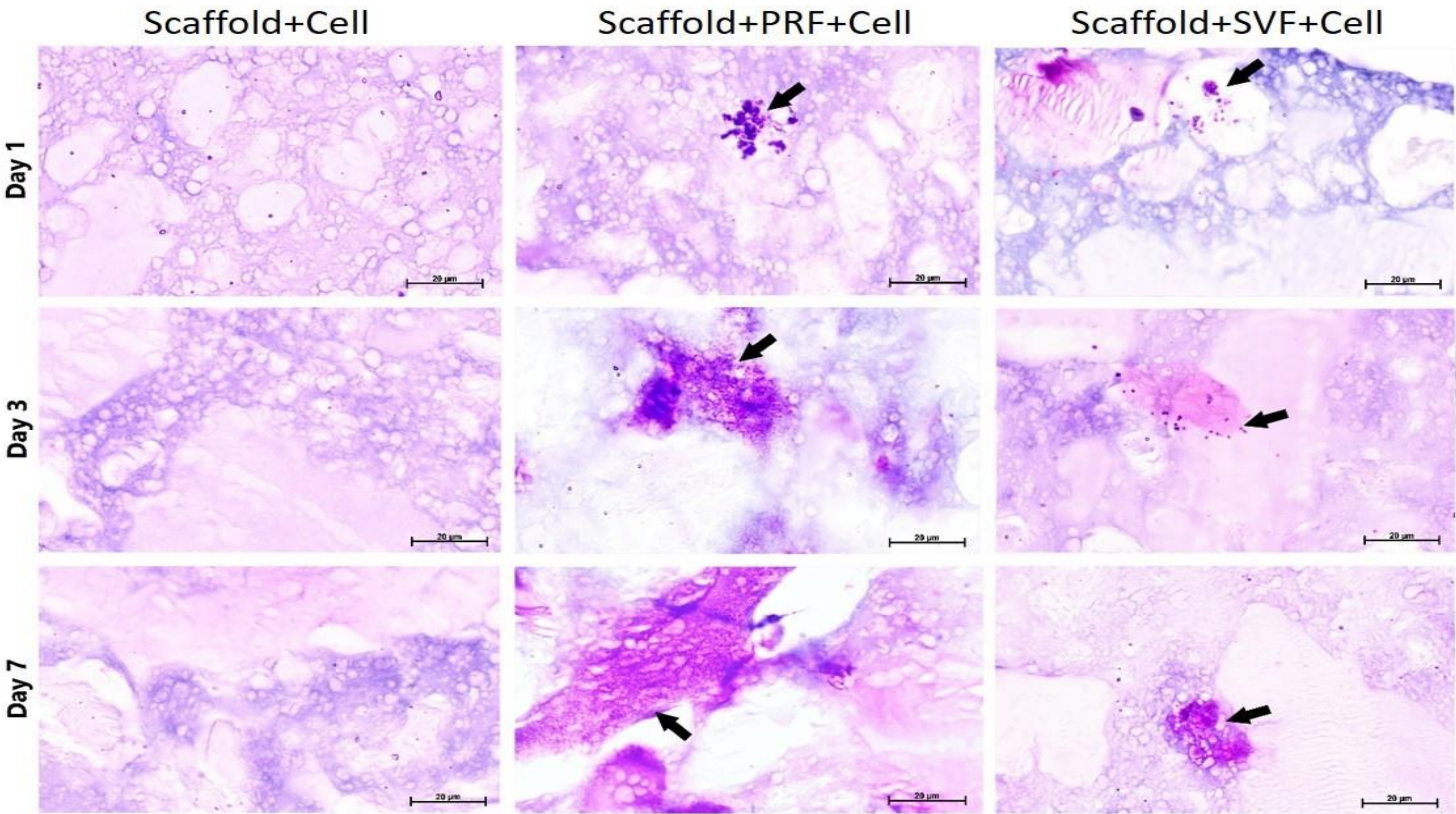


Figure 8. Giemsa staining on the first, third, and seventh days after cultivation. The black arrows point towards granular purple aggregates in the Scaffold+PRF+Cell and Scaffold+SVF+Cell groups.