Polycaprolactone–Gold Nanoparticle Scaffolds: Assessing Biocompatibility and Osteogenic Potential In Vitro

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Background

Tissue engineering aims to develop functional biomaterials that support bone regeneration by mimicking the extracellular matrix and promoting osteogenic differentiation. PCL-based scaffolds have gained attention due to their biocompatibility, biodegradability, and ability to enhance cell adhesion and proliferation. This study evaluates the viability of bmMSCs and the temporal expression of key osteogenic markers in these cells when seeded on PCL-based scaffolds with Gold Nanoparticles (AuNPs) of five different concentrations under osteogenic conditions, with an emphasis on their potential for bone regeneration. Understanding the expression of osteogenic markers in bmMSCs cultured on PCL scaffolds with AuNPs is essential for optimizing their application in bone tissue engineering.

Materials and Methods

Bone Marrow-derived Mesenchymal Stem Cells (bmMSCs) were seeded in scaffolds of seven different PCL and AuNPs concentrations and cultured in osteogenic induction medium containing platelet lysate, dexamethasone, ascorbic acid and β-glycerophosphate. Cell proliferation and viability analysis was performed with MTS test at days 1, 7, and 14. Alkaline phosphatase activity was measured spectrophotometrically at days 1, 3, 7, and 10. The expression levels of osteogenic markers, including Secreted Phosphoprotein 1 (SPP1) and collagen type I (COL1) were analysed using quantitative real-time PCR (qPCR). Fluorescence imaging, Osteocalcin and Collagen I stainings, were used to visualize cell morphology and protein expression over time.

Results

The metabolic activity and proliferation measurement indicates that the PCL scaffolds electrospun with EtOH instead of DMF have higher cell viability (PCL-EtOH-400mg/L). The ALP results presented in this study show that all PCL-based scaffolds promoted osteogenic differentiation and there were no significant differences. The expression levels of the osteogenic marker collagen type I, measured with qPCR, showed significantly higher expression in the scaffolds with the embedded AuNPs except for PCL-DMF-200mg/L which appears to be toxic. Similarly, the results regarding the expression of SPP1 show a significant difference in its expression in the same scaffolds. The cells seeded in the scaffolds PCL-EtOH-200/400mg/L and PCL-DMF-200mg/L present the highest osteogenic differentiation. On the same wavelength, the microscopic analysis for collagen I (Day 21) and Osteocalcin (Day 28) indicate active osteoblast differentiation and bone-like matrix deposition.

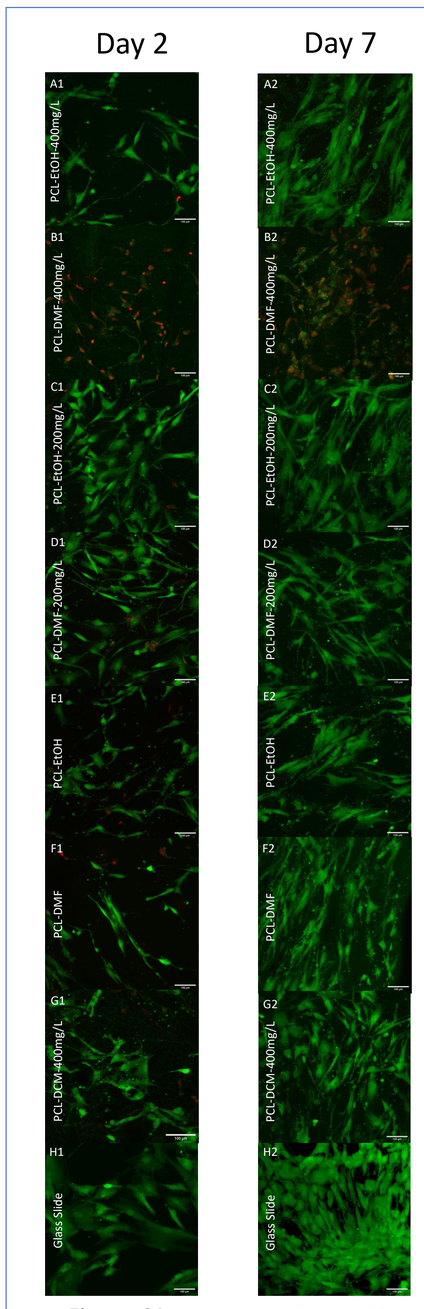


Figure 6A. *Figure 6B.*

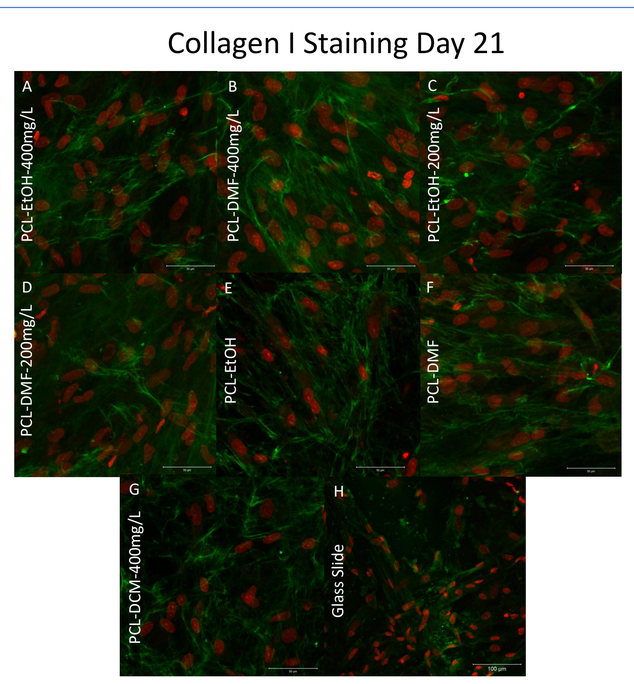


Figure 7.

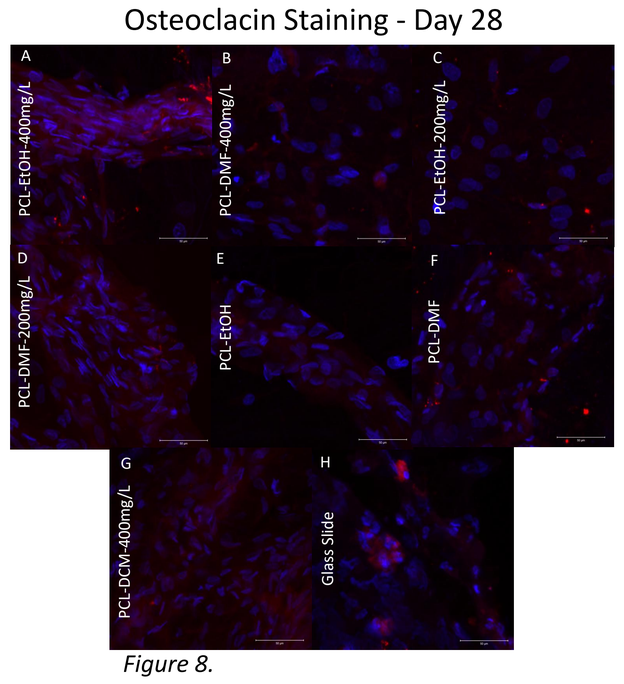


Figure 8.

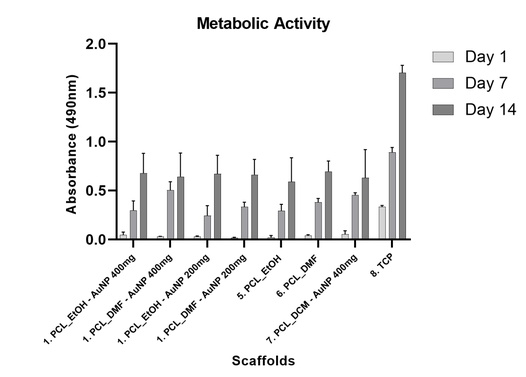


Figure 1. Metabolic Activity Measurement.

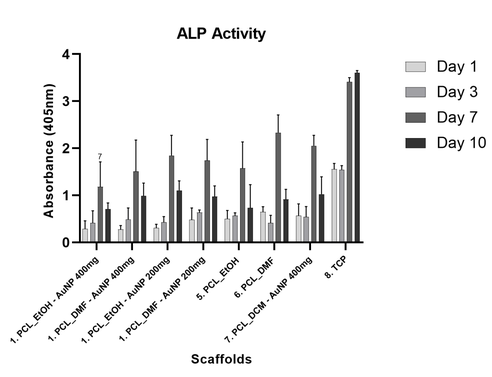


Figure 2. Alkaline Phosphatase Activity Measurement.

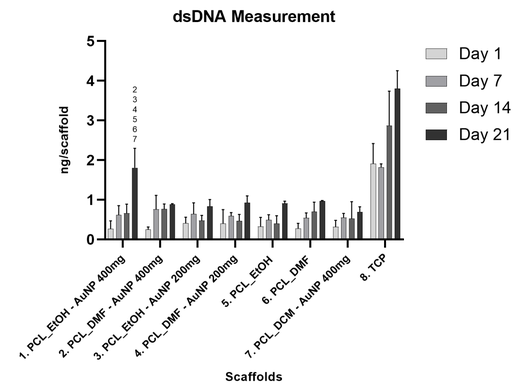


Figure 3. Proliferation of bmMSCs.

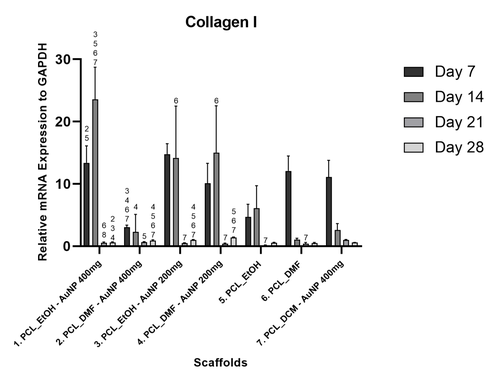


Figure 4. Relative Expression of Collagen I.

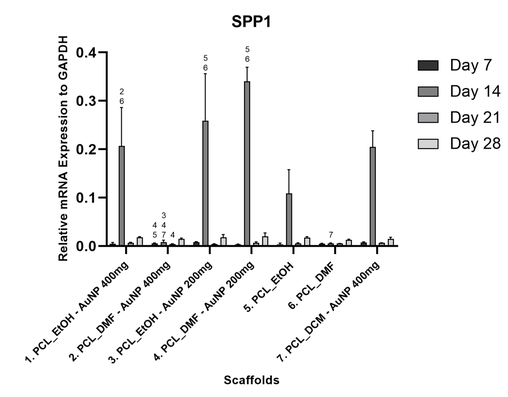


Figure 5. Relative Expression of SPP1.

Figure 1 & 3. Cell metabolic activity and proliferation of bmMSCs seeded on electrospun PCL-AuNP scaffolds. Absorbance measured at 480 nm and 523nm, respectively. Statistical significance marked with the number of each group for $p < 0.01$.
Figure 2. Alkaline Phosphatase activity of bmMSCS seeded on scaffolds. Absorbance measured at 405 nm. Statistical significance marked with the number of each group for $p < 0.01$.
Figure 4 & 5. Expression of Collagen I and SPP1 mRNA for days 7, 14, 21, and 28. Statistical significance marked with the number of each group for $p < 0.01$.

Figure 6A & 6B. Live-Dead Staining of bmMSCs. Emission in green visualize living cells. Emission in red visualize nuclei of dead cells. Scale bar 100 µm. (1) are images from day 2. (2) are images from day 7. Control group are cells seeded on glass slides.
Figure 7. Visualization of Collagen I on day 21 using immunohistochemistry. Red fluorescent nuclei indicate dead cells, green fluorescent dye indicates Collagen I. Scale bar 50 µm. Successful collagen I staining was evident through a clear, fibrillar pattern localized in the extracellular space surrounding the cells.
Figure 8. Visualization of Osteocalcin on day 28 using immunohistochemistry. Cell nuclei visualized with Hoechst, osteocalcin red. Scale bar 50 µm. Positive immunohistochemical staining for osteocalcin confirms successful detection of osteogenic marker expression in the samples.

Discussion

The temporal expression of osteogenic markers aligns with established osteogenesis stages, with ALP promoting early mineralization, and collagen I expression remaining consistently high throughout differentiation, emphasizing its crucial role in extracellular matrix formation and mineralization. Microscopic visualization confirmed scaffold-mediated morphological changes consistent with osteogenesis, establishing the role of PCL along with AuNPs in promoting bone tissue formation. Scaffolds with 400 mg/L and 200 mg/L of AuNPs, electrospun with EtOH, as well as the scaffold PCL-DMF-200mg/L of AuNPs seem to provide a suitable matrix for bmMSCs adhesion and differentiation. These findings support the application of these scaffolds in regenerative medicine and highlight the importance of optimizing scaffold composition to enhance osteogenic potential.

Conclusion

This study demonstrates the progressive expression of osteogenic markers in bmMSCs that were seeded on PCL-based scaffolds with AuNPs, confirming their differentiation potential. The integration of molecular, biochemical, and microscopic analyses strengthens our understanding of osteogenesis. These results contribute to the advancement of stem cell-based bone regeneration strategies and the development of optimized osteogenic induction protocols.

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