Incorporating Hydrophilic Polymers and Bioactive Nanoparticles into PCL for Advanced Bone Regeneration

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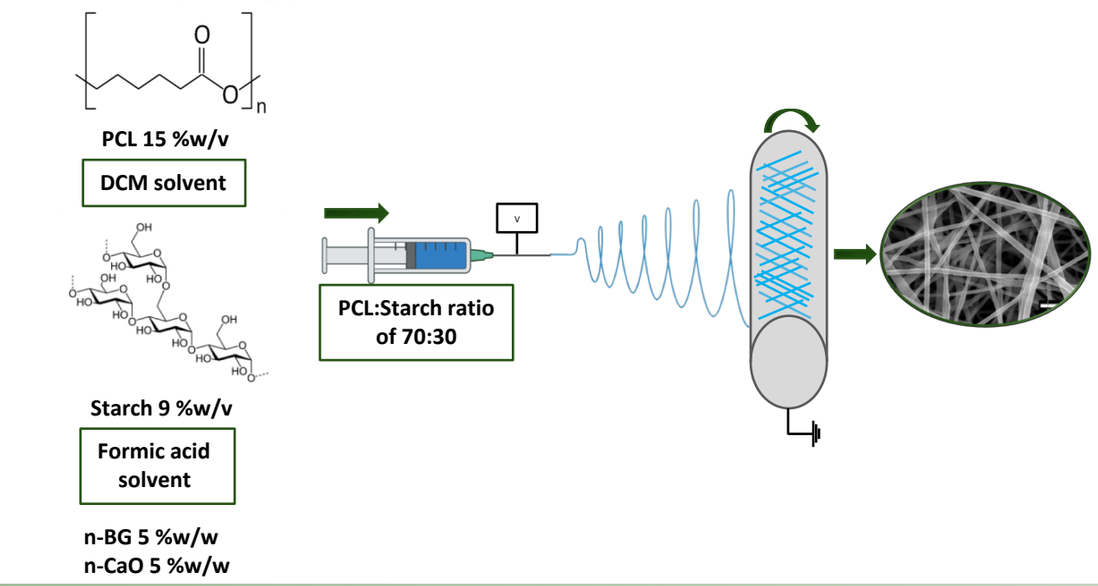
1. ABSTRACT

Bioactive electrospun scaffolds with enhanced biological and mechanical properties were developed using polycaprolactone (PCL) blended with starch and reinforced with bioactive nanoparticles (calcium oxide (CaO) and bioglass (BG)). PCL was chosen for its biocompatibility and mechanical properties [1], starch for its biodegradability and hydrophilicity [2], and nanoparticles for their potential to induce biomineralization [3,4,5]. The addition of CaO nanoparticles increased the average fiber diameter to approximately 1200 nm, while BG-reinforced fibers exhibited diameters ranging from 311 to 438 nm. Both scaffold types demonstrated high porosity, pore interconnectivity, and significant improvements in water absorption. Enhanced degradation rates were observed, with mass losses of 60% and 37% for PCL/Starch/CaO and PCL/Starch/BG scaffolds, respectively. Mechanical analysis revealed opposing trends: CaO incorporation increased Young's modulus by 60%, while BG reduced it by 52%. Both formulations exhibited biomineralization capacity, forming hydroxyapatite on their surfaces after immersion in simulated body fluid (See Figure 5). Biological evaluations demonstrated excellent cell adhesion and viability for preosteoblastic MC3T3-E1 and MG-63 osteoblast-like cells. In vivo studies using a subdermal Wistar rat model confirmed superior biocompatibility and bioresorbability, with enhanced healing and reabsorption compared to neat PCL. These findings highlight the synergistic effects of combining starch and bioactive nanoparticles in electrospun scaffolds, making PCL/Starch/CaO and PCL/Starch/BG composites promising candidates for bone tissue engineering applications.

2. EXPERIMENTAL METHODS

2.1. Electrospinning process.



3. RESULTS AND DISCUSSION

3.1. PCL, PCL/Starch scaffolds with incorporation of BG and CaO nanoparticles obtained by electrospinning technique.

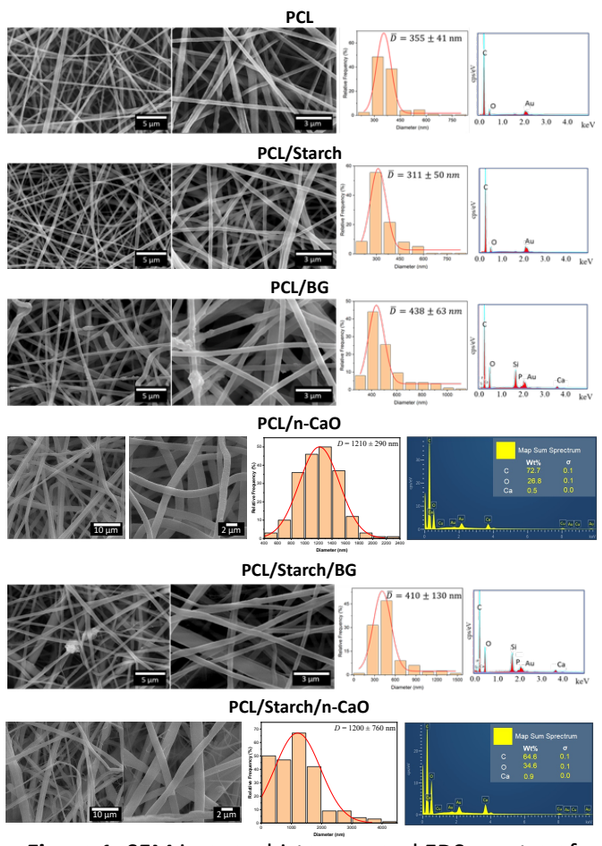


Figure 1. SEM images, histogram and EDS spectra of PCL, PCL/Starch, PCL/BG, PCL/n-CaO, PCL/Starch/BG and PCL/Starch/n-CaO fibers.

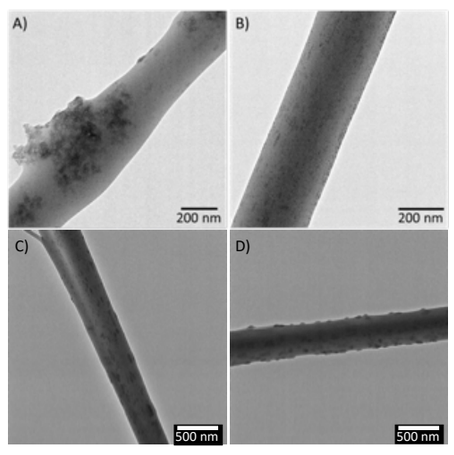


Figure 2. TEM images of electrospun fibers directly onto the TEM grid: A) PCL/BG, B) PCL/Starch/BG, C) PCL/n-CaO and D) PCL/Starch/n-CaO

3.2. Water absorption and degradability.

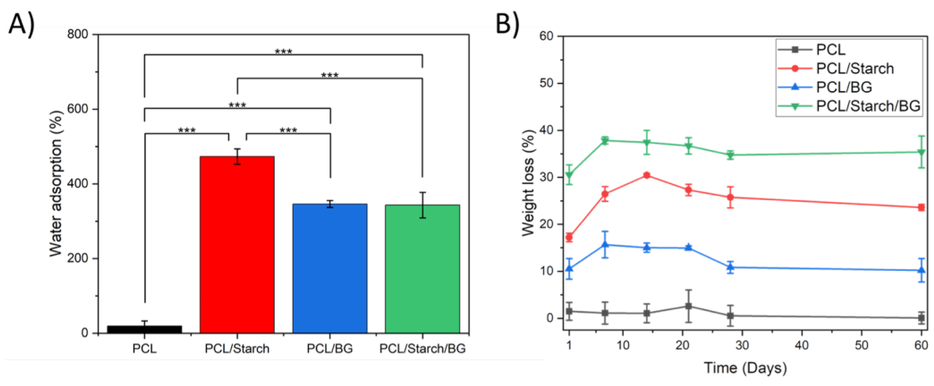


Figure 3. A) Water absorption after 24 hours and B) Degradability over 60 days in PBS solution for PCL, PCL/Starch, PCL/BG, and PCL/Starch/BG scaffolds.

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[1] Dias, J.R. et al. Electrospun Polycaprolactone (PCL) Degradation: An In Vitro and In Vivo Study, Polymers, 2022, 14, 16.
[2] Asl M.A., et al. Evaluation of the effects of starch on polyhydroxybutyrate electrospun scaffolds for bone tissue engineering applications, Int. J. Biol. Macromol. 2021, 19,1 500-513.
[3] D. A. Canales et al., Fabrication and assessment of bifunctional electrospun poly(l-lactic acid) scaffolds with bioglass and zinc oxide nanoparticles for bone tissue engineering, Int. J. Biol. Macromol., 2023, 228, 78-88.
[4] D. Canales, et al., Preparation and characterization of novel poly (lactic acid)/calcium oxide nanocomposites by electrospinning as a potential bone tissue scaffold, Biomater. Adv. 2023, 153, 213578.
[5] Garcia, G. et al. Electrospun scaffolds based on a PCL/starch blend reinforced with CaO nanoparticles for bone tissue engineering, Int. J. Biol. Macromol, 2024, 273, 132891.

3. RESULTS AND DISCUSSION

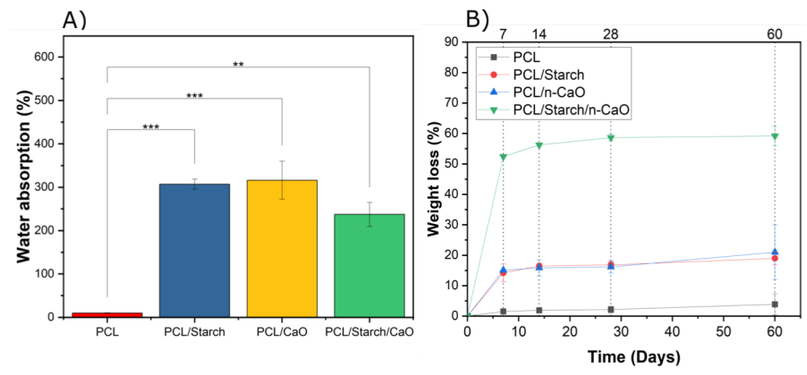

Figure 4. A) Water absorption after 7 days and B) Degradability over 60 days in PBS solution for PCL, PCL/Starch, PCL/n-CaO, and PCL/Starch/n-CaO scaffolds.

3.3. *In vitro* biomineralization analysis.

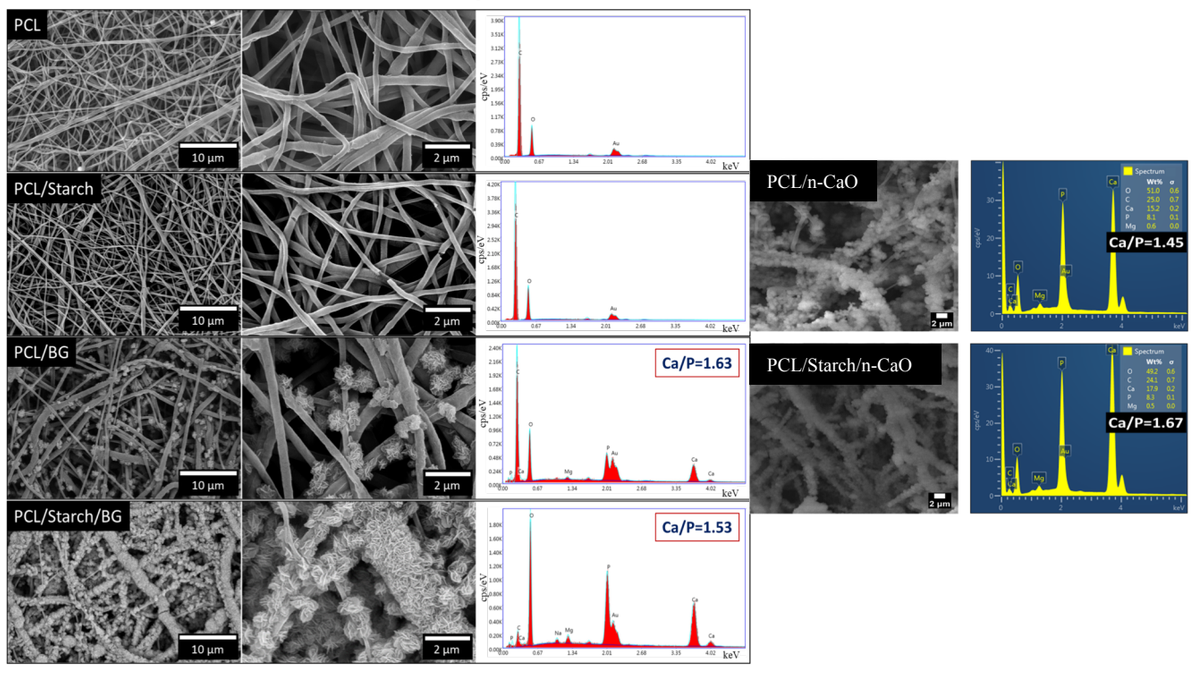

Figure 5. SEM images and EDS spectra of PCL, PCL/Starch, PCL/n-CaO, PCL/BG, PCL/Starch/BG and PCL/Starch/n-CaO scaffold at 14 days of immersion in SBF solution.

3.4. *In vitro* biological characterization.

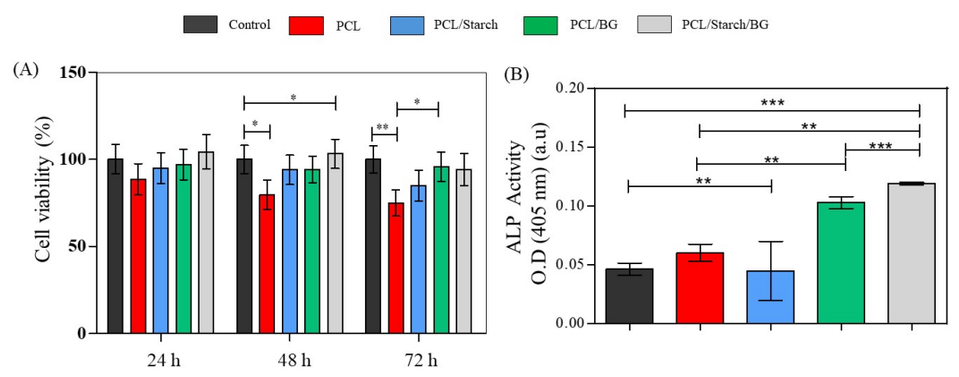

Figure 6. (A)Statistical analysis of cell viability , after 24, 48 and 72 hours of exposure, and (B) alkaline phosphatase (ALP) activity cultured in osteogenic medium for 7 days, both assays for PCL, PCL/Starch, PCL/BG and PCL/Starch/BG scaffolds using an MG-63 cell line. The activity of ALP was performed using pNPP as a substrate (n=3, *p<0.1, **p<0.01)

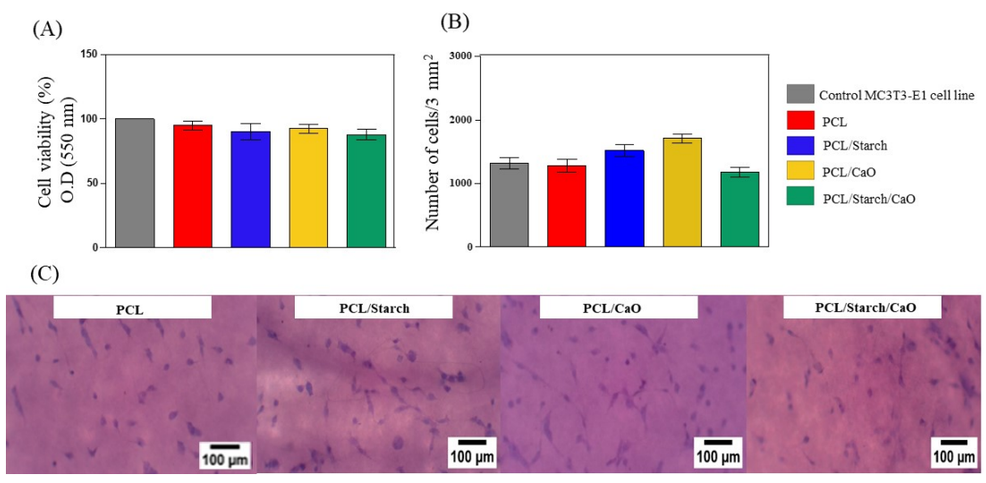

Figure 7. *In vitro* biological characterization of PCL-based scaffolds (A) Cell viability after 72 hours by MTT assays (B) cell adhesion after 48 hours using a trypan blues as a cell marker, and (C) Cell adhesion images by light microscopy. For Cell viability (n=6) and cell adhesion (n=3): * = p < 0.05, ** p < 0.01, *** = p < 0.001).

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4. CONCLUSION

Electrospun scaffolds based on PCL reinforced with starch and bioactive nanoparticles (CaO or bioglass) were successfully fabricated, exhibiting homogeneous fiber deposition with diameters ranging from 311 to 1200 nm depending on composition. The incorporation of starch improved nanoparticle dispersion and fiber morphology while significantly enhancing water absorption—up to 1700% in PCL/Starch/BG after 24 h and 3500% in PCL/Starch/CaO after 7 days—indicating improved hydrophilicity. Both scaffolds demonstrated increased degradation rates, with PCL/Starch/CaO showing ~60% mass loss over 60 days and PCL/Starch/BG reaching ~37% in shorter periods. Thermal analysis revealed that starch increased crystallinity, while CaO and BG decreased it; the combined system (PCL/Starch/BG) exhibited intermediate behavior, suggesting a compensatory effect of starch. Mechanically, PCL/Starch/CaO scaffolds exhibited a ~60% increase in Young's modulus but decreased tensile strength and elongation. In contrast, PCL/Starch/BG showed reductions in stiffness and strength but improved flexibility (~20% increase in elongation at break). Both systems supported hydroxyapatite (HA) formation on their surfaces—within 14 days for BG and 28 days for CaO—confirming their bioactivity. In vitro studies with MG-63 osteoblast-like cells demonstrated excellent adhesion, proliferation, and biocompatibility. In vivo subdermal implantation in Wistar rats confirmed scaffold resorption and integration, supporting their suitability for bone tissue applications.
Overall, both PCL/Starch/CaO and PCL/Starch/BG scaffolds exhibited promising properties for bone tissue engineering, combining bioactivity, biocompatibility, and biodegradability. Further optimization could tailor their performance for specific clinical needs.