Development of Polycaprolactone/Kefiran/n-CaO Biomaterials for Bone Regeneration

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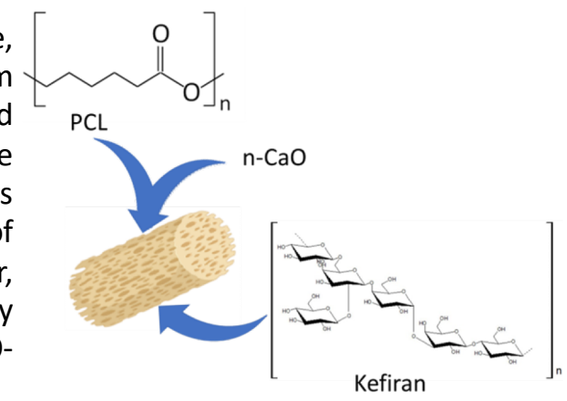
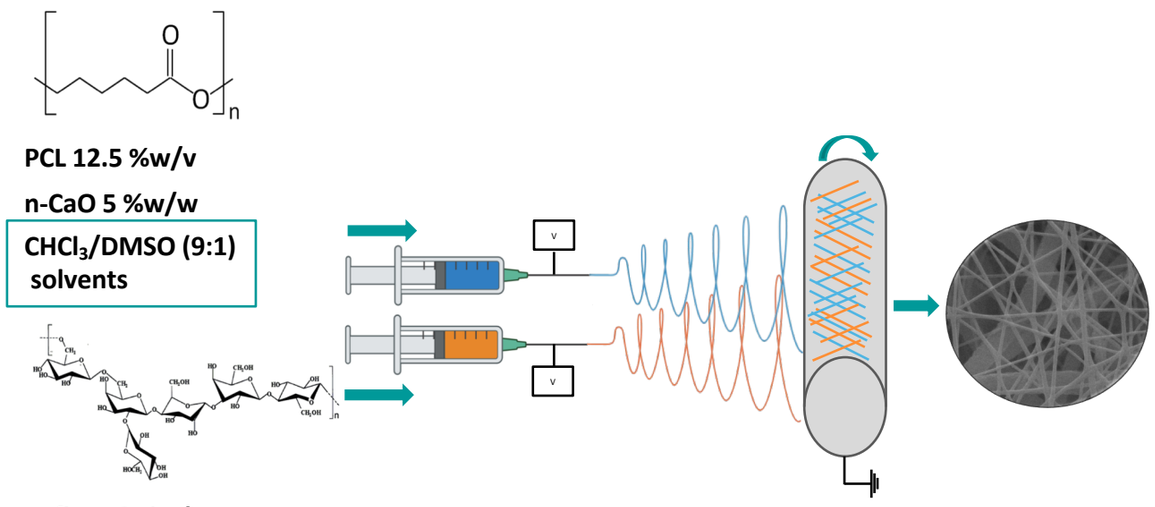
1. ABSTRACT

Among the biomaterials used in tissue engineering for scaffold development, polycaprolactone (PCL) stands out as a versatile, biodegradable synthetic polymer with good mechanical properties [1]. This work focuses on the fabrication of electrospun scaffolds from PCL combined with Kefiran, a natural biopolymer known for its high hydrophilicity, with the aim of improving material degradation and promoting cell adhesion in the tissue [2]. Calcium oxide nanoparticles (n-CaO) were incorporated due to their ability to stimulate the formation of hydroxyapatite (HA), a key component for bone tissue regeneration. The PCL/Kefiran/n-CaO scaffold presented homogeneous and defect-free fibers (Figure 1). After immersing the scaffolds in simulated body fluid (SBF) solution, it was observed that the addition of n-CaO promoted HA formation on the scaffold surface, and the presence of Kefiran further enhanced this formation (Figure 2). Moreover, the PCL/n-CaO and PCL/n-CaO-Kefiran scaffolds did not show cytotoxic effects on human osteosarcoma cells (MG-63), with a cell viability reduction of less than 30%, in accordance with ISO-10993-5 standards (Figure 5A). These results suggest that PCL/n-CaO and PCL/n-CaO-Kefiran scaffolds have great potential as devices for bone tissue regeneration.



2. EXPERIMENTAL METHODS

2.1. Electrospinning process



3. RESULTS AND DISCUSSION

3.1. PCL/n-CaO-Kefiran scaffolds obtained by electrospinning technique.

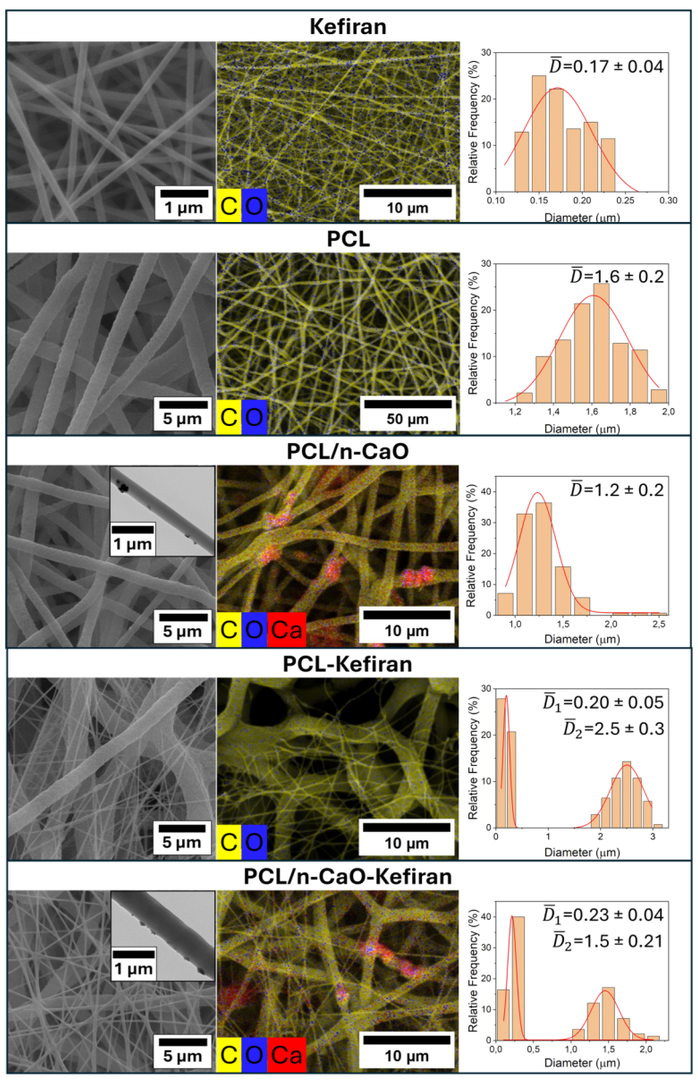


Figure 1. SEM micrographs, elemental mapping (EDS), and fiber diameter distributions of electrospun neat Kefiran, neat PCL, PCL/n-CaO, PCL-Kefiran, and PCL/n-CaO-Kefiran scaffolds. Insets show representative TEM images of fibers directly electrospun onto grids.

3.2. In vitro mineralization assay in simulated body fluid (SBF) at 28 days.

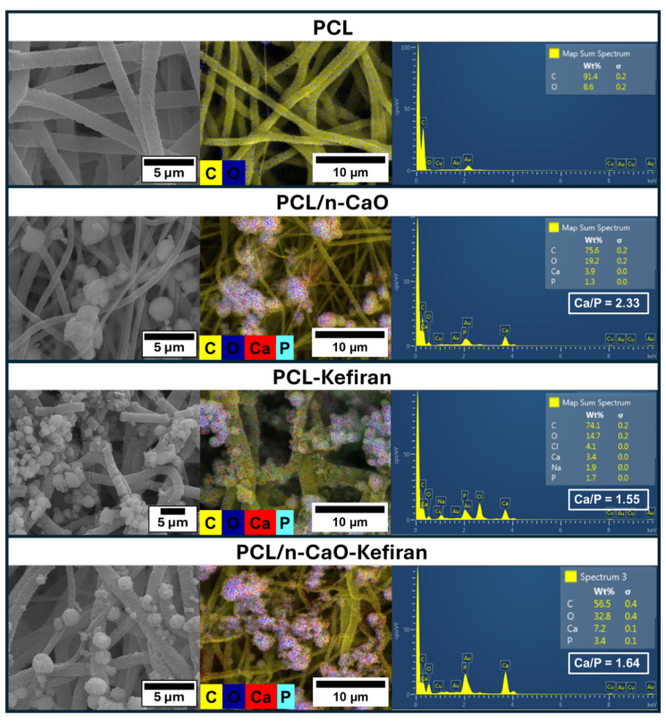


Figure 2. SEM images, EDS map and spectra of PCL, PCL/n-CaO, PCL-Kefiran, and PCL/n-CaO-Kefiran scaffolds at 28 days of immersion in SBF solution.

3. RESULTS AND DISCUSSION

3.3. Water absorption and degradability.

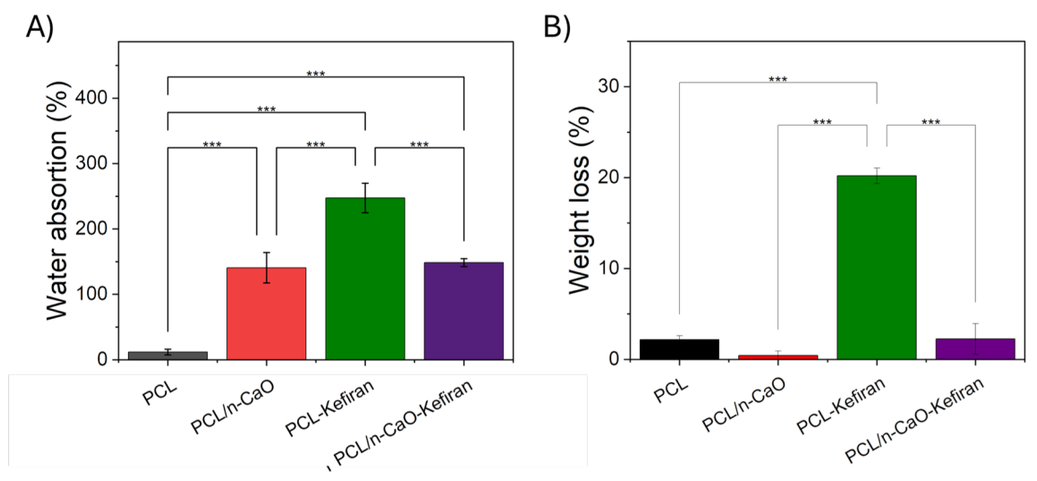


Figure 3. A) Water absorption after 24 hours and **B)** Degradability at 60 days in PBS solution for PCL, PCL/n-CaO, PCL-Kefiran, and PCL/n-CaO-Kefiran scaffolds. Bonferroni * p<=0,05 ** p<=0,01 *** p<=0,001

3.4. Mechanical properties of electrospun scaffolds by uniaxial tensile test.

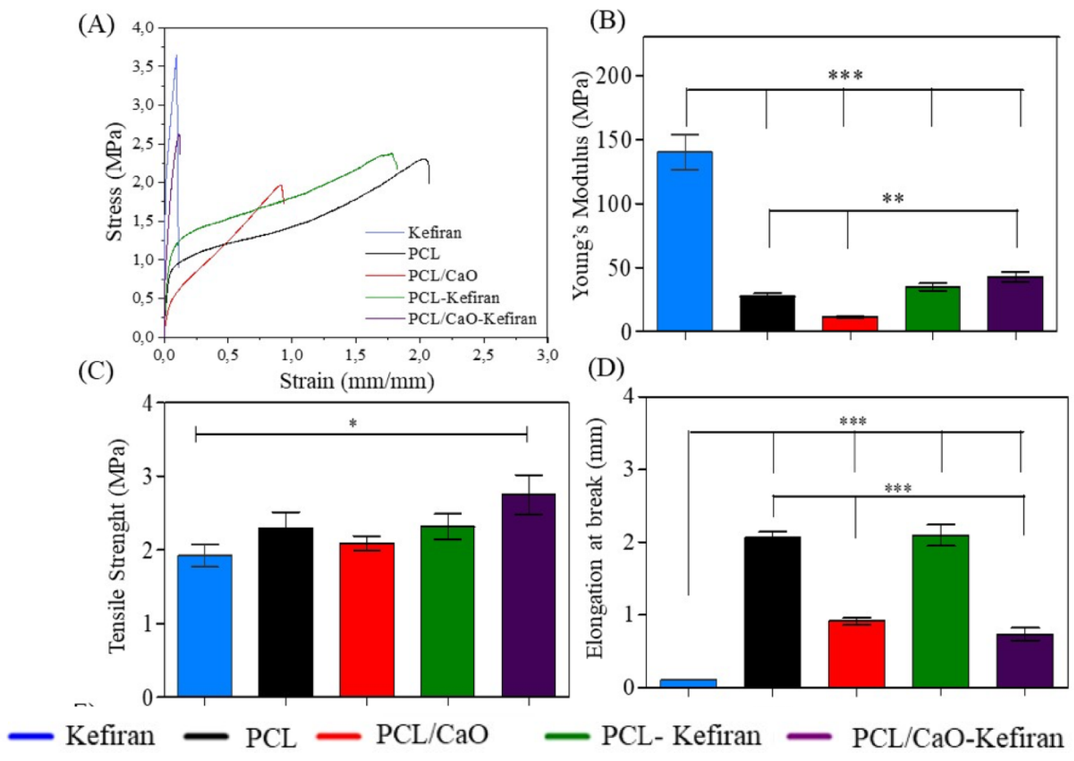


Figure 4. A) Stress-strain diagram, **B)** Young´s modulus, **C)** Tensile strength, **D)** elongation at break (n=7, **= p < 0.01 and *= p < 0.05), for PCL, PCL/n-CaO, PCL-Kefiran, and PCL/n-CaO-Kefiran scaffolds.

3.5. Cell viability assay and alizarin red-based mineralization assay.

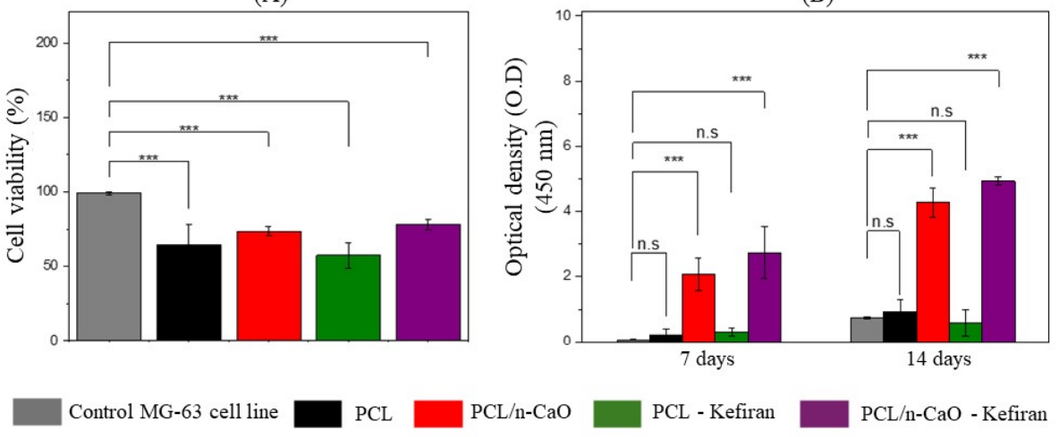


Figure 5: **a)** Viability of MG-63 cells determined by MTS assay at 24 hours, **b)** alizarin red-based mineralization assay at 7 and 14 days for scaffolds composed of PCL, PCL/n-CaO, PCL-Kefiran and PCL/n-CaO-Kefiran. *** denotes a significant difference with a value of p ≤ 0,001.

ACKNOWLEDGMENTS

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4. CONCLUSION

Bioactive scaffolds for bone tissue engineering were successfully developed using double-jet electrospinning. Scaffolds presented a fibrous, porous, and highly interconnected structure. PCL scaffolds had uniform fibers, while PCL-Kefiran and PCL/n-CaO-Kefiran scaffolds combined micrometer-sized PCL or PCL/n-CaO fibers with nanometer-sized Kefiran fibers. The presence of Kefiran improved surface hydrophilicity and accelerated degradation. In vitro mineralization confirmed hydroxyapatite formation on PCL/n-CaO, PCL-Kefiran, and PCL/n-CaO-Kefiran after 28 days in SBF, supported by FTIR-ATR, XRD, and SEM/EDS, which revealed Ca/P molar ratios (1.5–2.3) consistent with bone-like apatite. Mechanically, PCL offered good strength; Kefiran improved stiffness, while n-CaO reduced strength but promoted mineralization. PCL/n-CaO and PCL/n-CaO-Kefiran scaffolds showed better biocompatibility, possibly due to calcium ion release, and demonstrated higher osteogenic potential by alizarin red staining. These systems are promising for bone regeneration applications.