Aptamer-enhanced SF/β-TCP/PCL scaffolds: sustainable innovation for craniofacial bone healing

Benedetta Ghezzi[1,2*], Biagio Matera[1], Giuseppe De Giorgio[2], Gianluca Turco[3], Katia Rupel[3], Barbara Medagli[3], Pasquale D'Angelo[2], Giuseppe Tarabella[2], Maddalena Manfredi[1]

1 Center of Dental Medicine, Department of Medicine and Surgery, University of Parma, Via Gramsci 14/A, 43126 Parma, Italy
2 IMEM-CNR, Institute of Materials for Electronics and Magnetism-National Research Council, Parco Area delle Scienze 37/A, 43124 Parma, Italy.
3 Clinical Department of Medical, Surgical and Health Sciences, University of Trieste, Piazza dell'Ospitale 1, 34129 Trieste, Italy
*Corresponding Author E-mail: benedetta.ghezzi@unipr.it

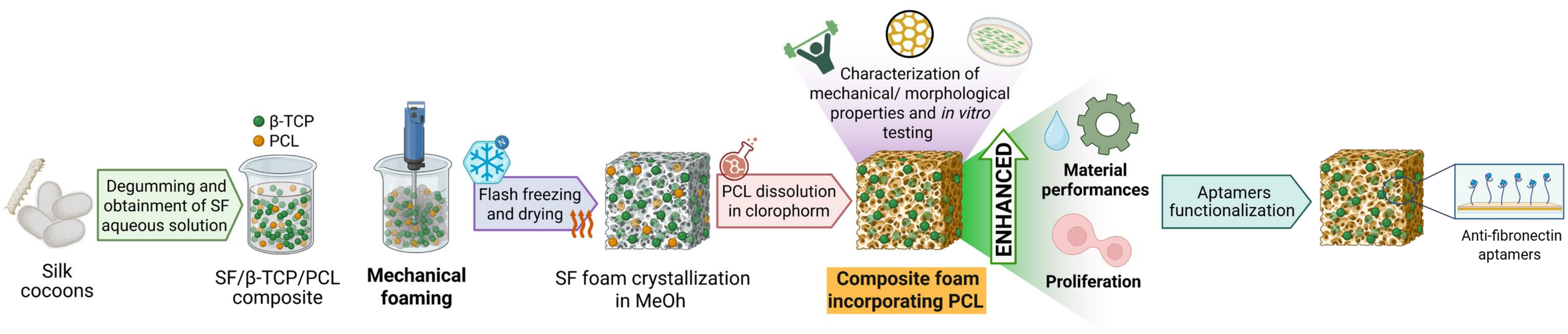
INTRODUCTION

Silk fibroin (SF) has gained increasing attention as a biomaterial for bone tissue engineering due to its excellent biocompatibility, biodegradability, and structural adaptability[1]. However, its limited mechanical strength and insufficient osteoconductive properties restrict its effectiveness in load-bearing and complex bone regeneration applications. To overcome these challenges, composite strategies that combine SF with other bioactive and mechanically robust materials are being actively explored[2]. In this context, the functionalization of scaffold surfaces with aptamers represents a promising approach to enhance cellular interactions and promote targeted biological responses.

OBJECTIVE

The objective of this project is to develop and characterize an innovative composite scaffold for craniofacial bone regeneration by integrating silk fibroin with poly-ε-caprolactone (PCL) and high percentages of β-tricalcium phosphate (β-TCP), with the final aim of further enhancing its biofunctionality through surface-bound anti-fibronectin aptamers. This strategy aims to improve the scaffold's mechanical performance, osteoconductivity, and biological specificity, while leveraging aptamer-mediated surface functionalization to stimulate cell adhesion, proliferation, and osteogenic differentiation, ultimately promoting efficient bone repair and regeneration.

MATERIALS AND METHODS



MECHANICAL ANALYSIS

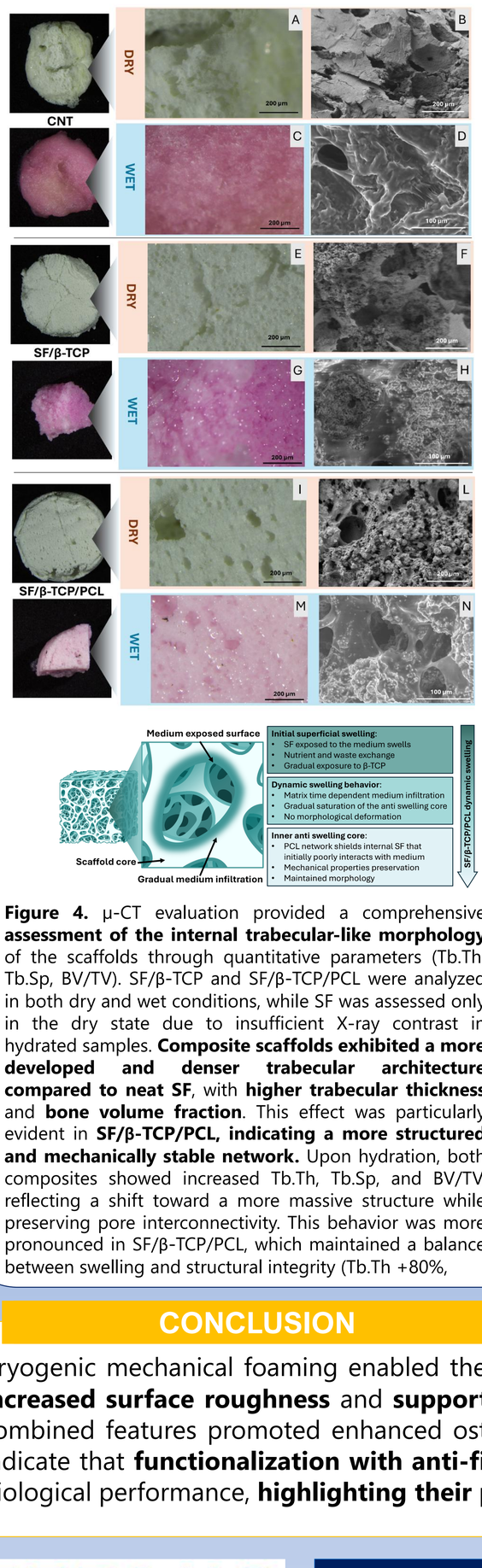


Figure 1. Overall **morphological analysis** of the **samples under dry and wet conditions** (stereoscopic images and ESEM micrographs). Morphological analysis confirmed that all scaffolds **exhibited porous and interconnected structures**, essential for tissue regeneration. **SF/β-TCP/PCL showed the best performance, maintaining high porosity (20–200 µm) and structural integrity in both dry and wet conditions.** SF/β-TCP preserved morphology after swelling, with moderate pore size reconfiguration. In contrast, pure SF (CNT) exhibited excessive swelling, leading to reduced pore size and compromised structure. Overall, PCL and β-TCP improved scaffold stability under physiological conditions, making SF/β-TCP/PCL the most promising formulation.

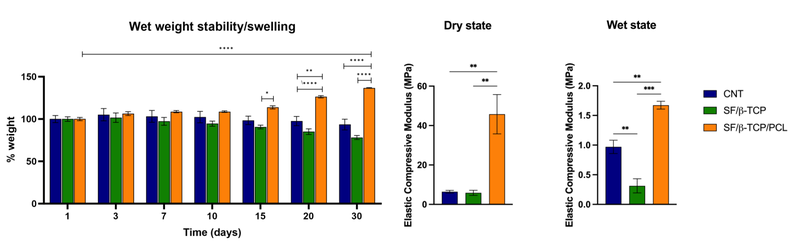
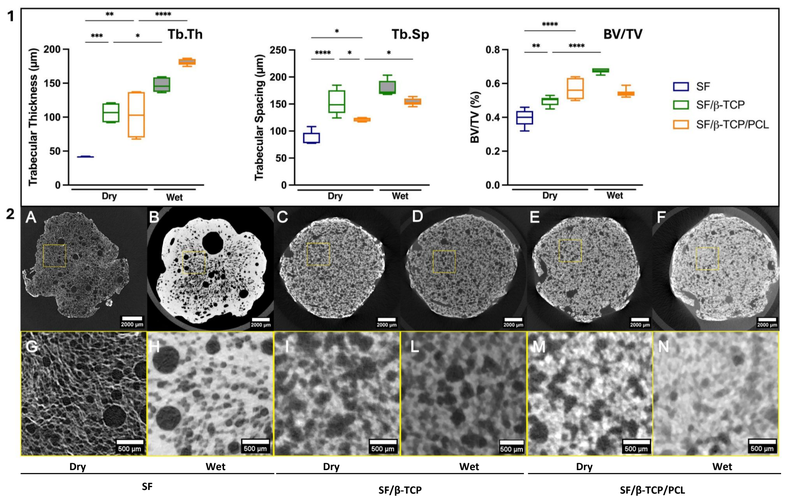


Figure 2. Analysis of wet weight stability/swelling monitoring performed over 30 days and the elastic compressive modulus on dry and wet samples. Swelling analysis showed that **β-TCP and especially PCL significantly reduced initial water uptake (Sr down to 18%)**, limiting excessive swelling. Conversely, higher Rw values **confirmed preserved porosity and surface hydration**, enabling fluid exchange. Over 30 days in PBS, **SF/β-TCP/PCL** scaffolds exhibited a **progressive weight increase, indicating a dynamic swelling behavior driven by medium infiltration and partial SF degradation.** This effect balances structural stability with gradual water absorption, **without significant deformation.** Overall, SF/β-TCP/PCL combines anti-swelling properties with adaptive behavior, supporting mechanical integrity and potential for tissue regeneration.

Figure 3. Schematic summary of SF/β-TCP/PCL samples dynamic swelling properties.

Figure 4. µ-CT evaluation provided a comprehensive **assessment of the internal trabecular-like morphology** of the scaffolds through quantitative parameters (Tb.Th, Tb.Sp, BV/TV). SF/β-TCP and SF/β-TCP/PCL were analyzed in both dry and wet conditions, while SF was assessed only in the dry state due to insufficient X-ray contrast in hydrated samples. **Composite scaffolds exhibited a more developed and denser trabecular architecture compared to neat SF**, with **higher trabecular thickness** and **bone volume fraction**. This effect was particularly evident in **SF/β-TCP/PCL, indicating a more structured and mechanically stable network.** Upon hydration, both composites showed increased Tb.Th, Tb.Sp, and BV/TV, reflecting a shift toward a more massive structure while preserving pore interconnectivity. This behavior was more pronounced in SF/β-TCP/PCL, which maintained a balance between swelling and structural integrity (Tb.Th +80%, Tb.Sp +41%).Overall, **SF/β-TCP/PCL scaffolds demonstrated enhanced structural stability, preserved porosity, and adaptive wet-state behavior**, highlighting their strong potential for bone regeneration applications.



BIOLOGICAL FEATURES

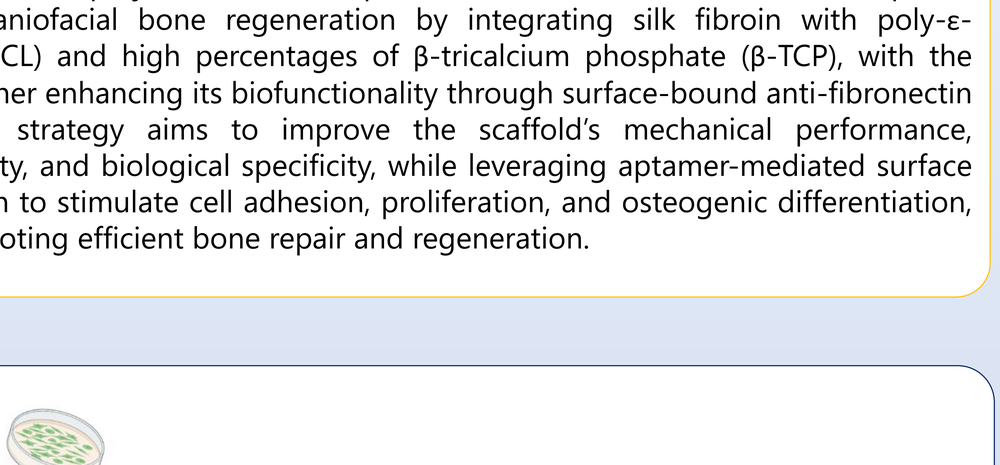
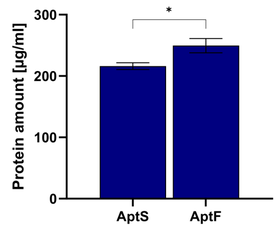


Figure 5. Biocompatibility, cell morphology and proliferation on fibroin-based scaffolds. (A) MTT stereoscope images revealed **homogeneous formazan distribution, indicating metabolically active cells,** while confocal imaging showed **well-spread cytoskeletons and morphologies** consistent with strong adhesion. **SF/β-TCP/PCL scaffolds supported higher viability and proliferation** compared to CNT, likely due to improved structural stability and nutrient exchange. Cell behavior may also be influenced by substrate stiffness via mechanotransduction pathways. (B) Indirect cytotoxicity assays using conditioned media (1, 7, and 10 days) showed **no cytotoxic effects**, with cell viability consistently above the 70% biocompatibility threshold, confirming the safety of the biomaterials and the absence of toxic by-products from the fabrication process.

APTAMER FUNCTIONALIZATION

Figure 6. Protein adsorption assay on SF/β-TCP/PCL scaffolds coated with **anti-fibronectin (AptF) and scrambled (AptS) aptamers**. The quantification of **human fibronectin binding** on AptS and AptF samples shows that **AptF exhibits a higher level** (p=0,04) of protein adsorption compared to AptS, indicating **increased surface affinity** for proteins under the tested conditions.



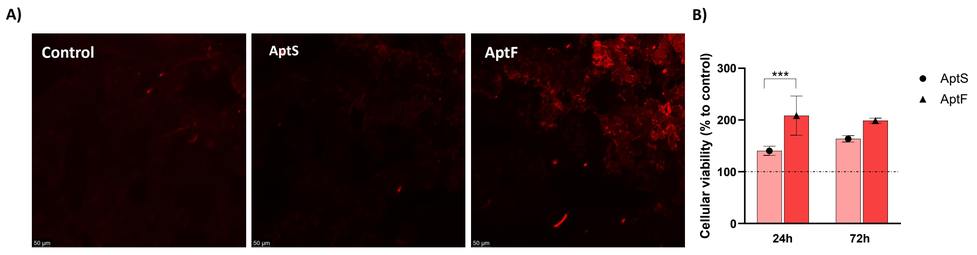


Figure 7. Representative fluorescence images (A) showing human fibronectin binding signal intensity on SF/β-TCP/PCL scaffolds for Control, AptS, and AptF conditions. **AptF displays a visibly increased fluorescence signal compared to Control and AptS**. Scale bar: 50 µm. Osteoblastic viability evaluation (B) confirms a **higher number of viable cells on the scaffolds coated with AptF after 24h** from cellular seeding, while AptS shows a modest change if compared to Control (dotted line). Anti-fibronectin aptamer functionalization of fibroin-based biomaterials shows **promising potential to enhance osteoblastic proliferation**.

CONCLUSION

Cryogenic mechanical foaming enabled the fabrication of highly porous silk fibroin-based scaffolds with **successful incorporation of β-TCP (30% w/v) and PCL (10% w/v)**. β-TCP **increased surface roughness** and **supported cell attachment**, while **PCL improved mechanical properties, structural stability, and induced dynamic swelling behavior**. These combined features promoted enhanced osteoblast proliferation, although further mechanical optimization is required to meet alveolar bone demands. Notably, preliminary findings indicate that **functionalization with anti-fibronectin aptamers further enhances osteoblastic proliferation.** Overall, these scaffolds exhibit high porosity, bioactivity, and improved biological performance, **highlighting their potential for bone and periodontal regeneration**.

REFERENCES

1. De Giorgio G, et al., Silk Fibroin Materials: Biomedical Applications and Perspectives. Bioengineering (Basel). 2024 Feb 9;11(2):167. DOI: 10.3390/bioengineering11020167
2. Matera B. et al., Silk-fibroin scaffolds enriched with calcium-based fillers for bone regeneration: a systematic review, Ceramics International, Volume 52, Issue 9, Part B, 2026, Pages 12313-12342, ISSN 0272-8842, DOI: 10.1016/j.ceramint.2026.01.443

FUNDING

The present work has been funded with the PRIN project: progetti di ricerca di rilevante interesse nazionale—Bando PRIN 2022 PNRR N. P2022NC9NT CUP: D53D23016540001 – PI Prof. Benedetta Ghezzi