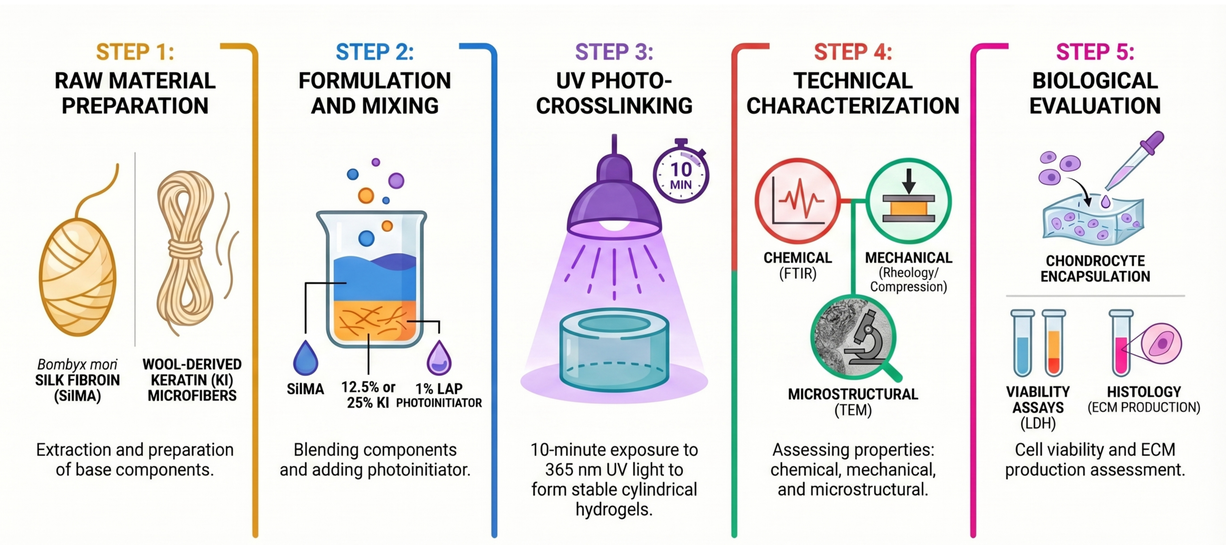
Silk fibroin methacrylate–keratin composite hydrogels for acetabular labrum regeneration

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Introduction

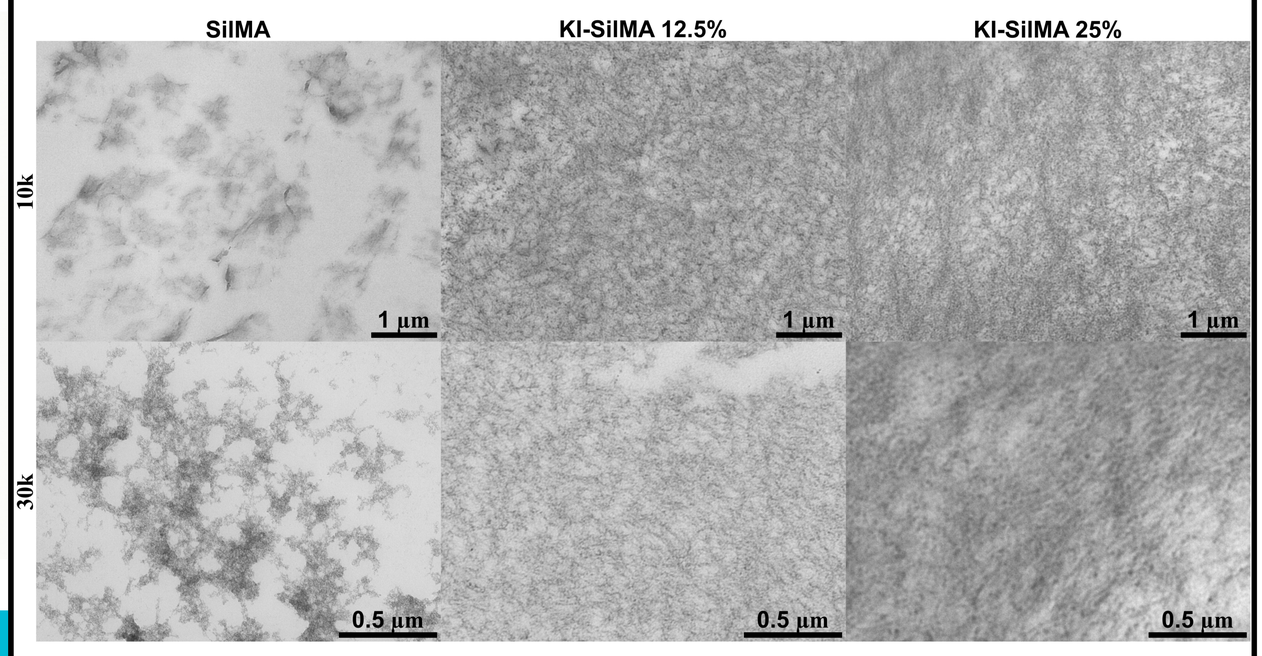
Human decellularized bone particles (dbPTs) are commonly used in clinical practice to perform allograft surgeries. To reduce the immunological response in the recipient and ensure their safety, these fragments, undergo to decellularization, a procedure that greatly reduces their osteogenicity. In this work, by including an ultrasonication step to further fragment the human bone, control dbPTs size distribution, tuning their osteogenic potential. A Response Surface Method (RSM) was used to develop equations able to predict dbPTs properties based on different ultasonication factors (power, duty cycle and time).

Methods



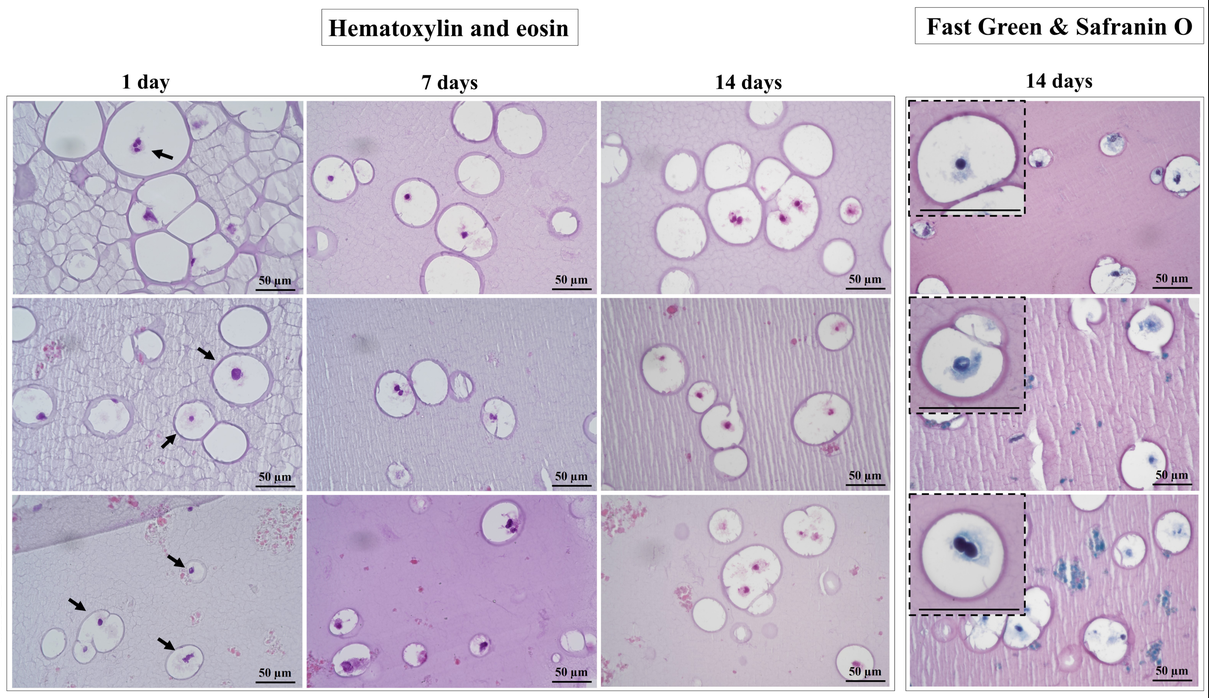
Silk fibroin was degummed from Bombyx mori cocoons and methacrylated with glycidyl methacrylate to produce SilMA. This matrix was blended with 12.5% or 25% w/w native keratin microfibers (KI), mixed with 1% LAP photoinitiator, and photo-crosslinked under 365 nm UV light for 10 minutes. Characterization included ATR-FTIR for chemical structure, rheology for viscosity, and unconfined compression for elastic modulus . For biological evaluation, human chondrocytes (C-28/I2) were encapsulated (15×10^6 cells/500 µl) to monitor viability (LDH assay) and ECM production (H&E, Safranin-O, and TEM) over a 14-day culture period.

Microstructure



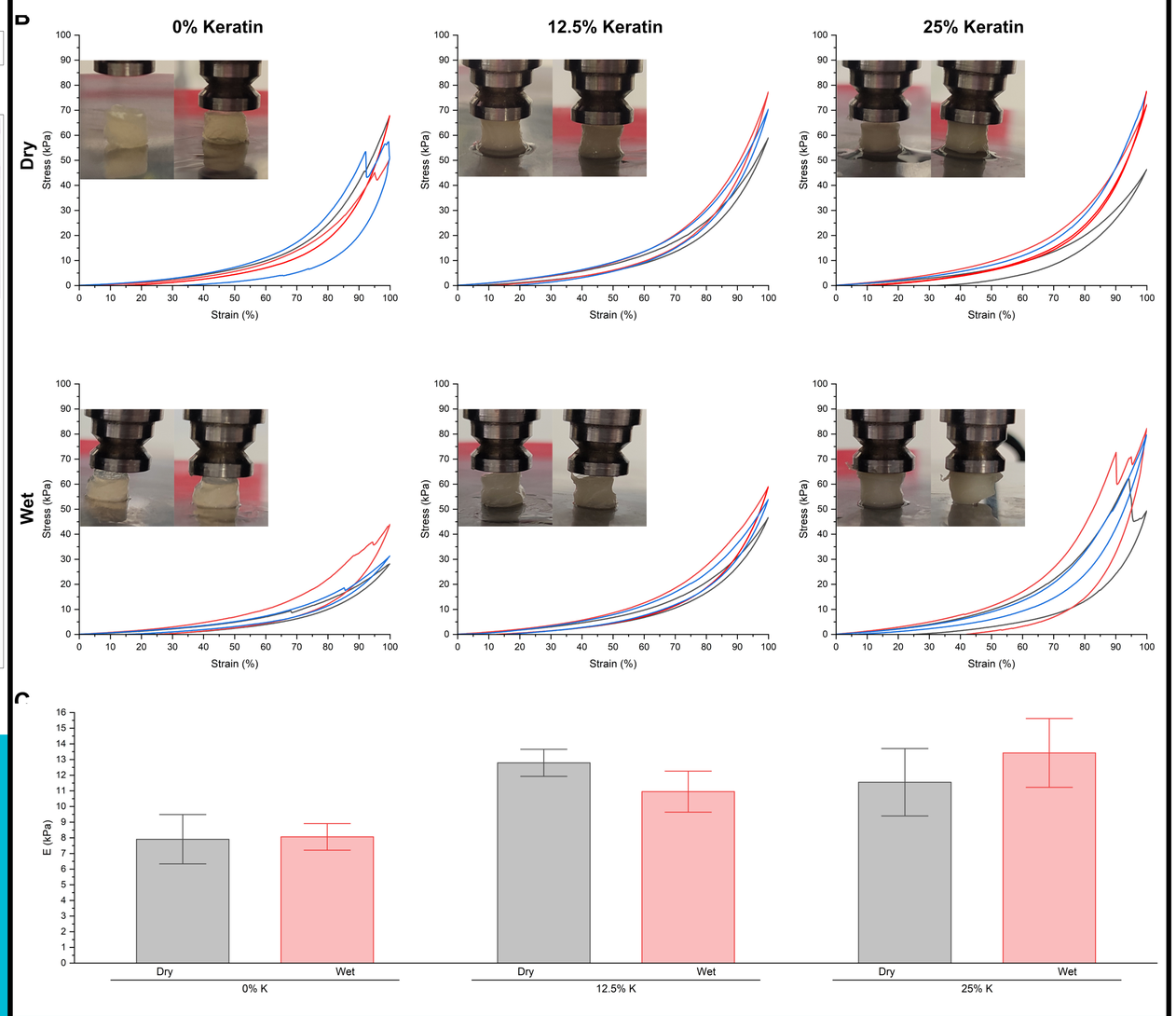
Microstructural analysis was performed using transmission electron microscopy (TEM) to evaluate the morphological organization of the hydrogels. The results revealed that keratin microfibers (KI) were uniformly distributed as darker inclusions within the brighter silk fibroin (SilMA) matrix, demonstrating stable integration and excellent homogeneity without macroscopic phase separation. Furthermore, high-resolution imaging after 14 days of culture confirmed the presence of granular proteoglycans, indicating early extracellular matrix production around the encapsulated cells

Histological Characterization



Hematoxylin and Eosin (H&E) staining confirmed that human chondrocytes were successfully embedded and distributed within the characteristic bubble-like structures of the hydrogel matrix throughout the study. By day 14, Safranin-O-Fast Green staining revealed discrete positively stained spots, providing evidence of early extracellular matrix (ECM) production and proteoglycan deposition. These histological findings, complemented by high-resolution TEM analysis, demonstrate that the composite hydrogel supports chondrocyte biosynthetic activity, with the most notable proteoglycan generation observed in the SilMA-KI25 formulation

Mechanical Characterization



The incorporation of keratin significantly improved the stiffness and mechanical integrity of the hydrogels in both dry and wet states. Keratin concentration was identified as the primary factor influencing the elastic modulus, the hydration state did not significantly impact the results. All formulations exhibited energy dissipation through a hysteresis loop and recovered their original shape after compression cycles. This energy dissipation is attributable to a viscoelastic behaviour of the integrated protein network, which allows the scaffold to withstand cyclic mechanical stress while maintaining its structural stability

Take home messages

- **This study marks the first development of a SilMA-KI composite hydrogel (silk fibroin and native keratin) specifically designed for acetabular labrum regeneration.**
- **The addition of keratin significantly increases the stiffness and viscosity of the scaffold, maintaining these improved properties in both dry and physiological conditions.**
- **The matrix is non-cytotoxic, ensuring high human chondrocyte viability for more than 14 days.**
- **The composite supports biosynthetic activity, as evidenced by early extracellular matrix (ECM) production and proteoglycan deposition**

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