Nanomechanical Characterization and Evaluation of Silk Fibroin Electrospun Scaffolds for Regenerative-Engineered Orthopedic Products

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Introduction/ Background

- Growing interest in biomanufacturing 3D living tissue and organs for regenerative medicine, leveraging superior mimicry of human physiology, supported by research evidencing improved replication of tissue architecture, with emphasis on mimicking the ECM through nano-scaled biopolymer fibers in 3D scaffold environments.
- Silk fibroin (SF), a natural biopolymer found in silkworm cocoons, possesses excellent mechanical properties due to its embedded nano-fibrillar structure, contributing to a balance of strength, modulus, toughness, extensibility, lightweight, and flexibility.
- **Research Objective:**
  - Performing nanomechanical tests on electrospun-silk fibroin nanofibers via Atomic Force Microscopy is proposed to accurately quantify load-strain behavior and understand their mechanical interaction with anchorage-dependent cells on electrospun substrates.

Fig 1. Applications of Silk Fibroin

Fig 9. Bruker MultiMode 8

Methods

Atomic Force Microscopy

- **Atomic Force Microscopy (AFM)** is a powerful tool used to image surfaces at the atomic level, providing high-resolution topographical information.
- F=-kz-cz - Hooke's Law with energy loss term.
- It relies on a small cantilever, featuring a sharp tip positioned extremely close to the sample surface, the cantilever scans across it, experiencing deflections due to interactions between the tip and the surface. The analysis is then performed using Nanoscope software.

Nanoindentation

- Nanoindentation measures mechanical properties by applying controlled force to deform a sample surface, enabling determination of properties like hardness and modulus by analyzing the force-depth curve. This is performed via tapping mode (externally oscillating the cantilever at its fundamental resonance frequency while scanning the surface.)
- Young's modulus can be determined through analytical models such as the JKR model or Hertzian contact theory, which relate the contact mechanics of the indenter with the sample's properties.

Fig 13. Image of probe with the cantilever

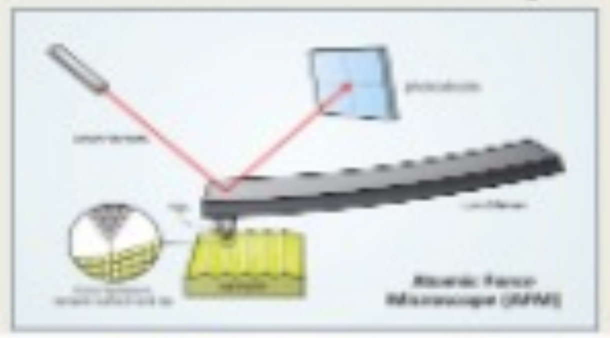

Fig 2. Atomic Force Microscopy-Components

Theory of Nanoindentation Mechanics

$$E = \frac{3}{4}(1-v)^2 \left\{\frac{1+16^{1/3}}{3}\right\}^{3/2} \frac{F_{min}}{\left(\pi(Sep_{zero} - Sep_{min})^3\right)^{1/2}}$$

Fig 11. The JKR Model

The Johnson-Kendall-Roberts (JKR) model, as depicted in Figure 1c, is particularly applicable to soft materials characterized by a Young's modulus < 1 GPa and exhibiting strong adhesion properties. Notably, the JKR model exclusively considers adhesion forces at the point of contact. Here, E represents Young's modulus, v denotes Poisson's ratio, F min signifies the minimum force at z = Sep_min, and Sep_zero designates the distance at which the force becomes zero during retraction.

Acknowledgements
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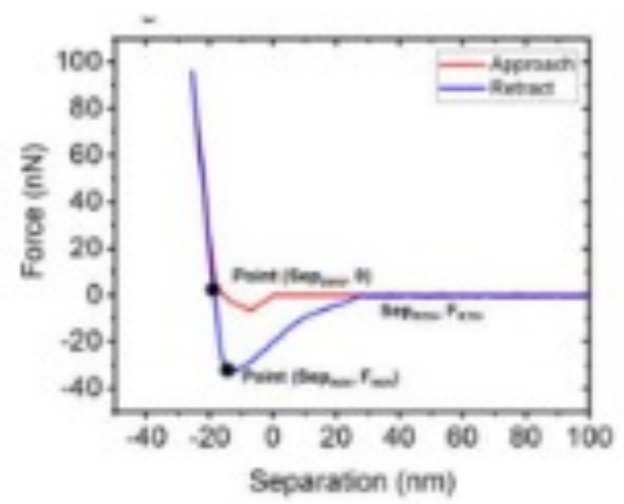


Fig 12. Experimental force–separation curves based on JKR Model

Experimental Results

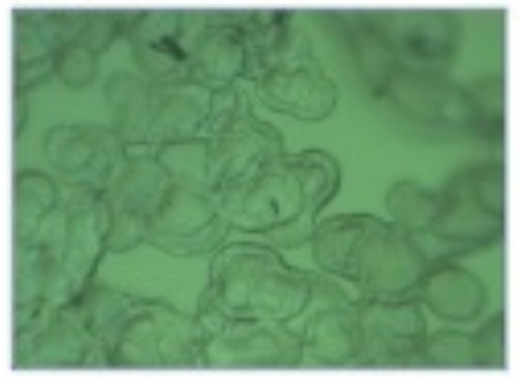
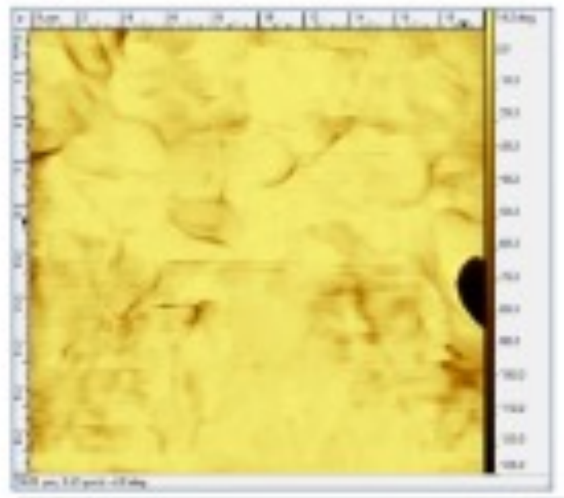
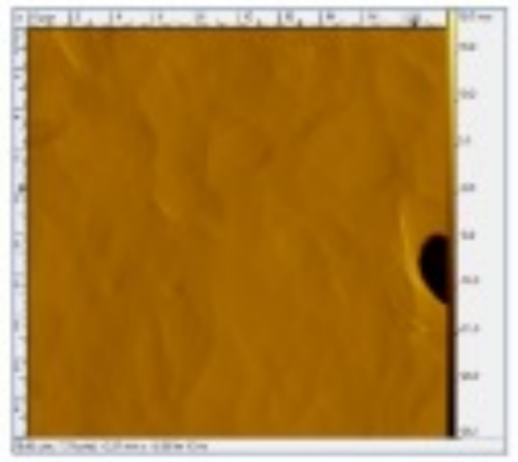


Fig 10. Optical Microscopy of electrospun Silk fibroin (100x)



(19.08 µm,8.65 µm): -4.0 deg

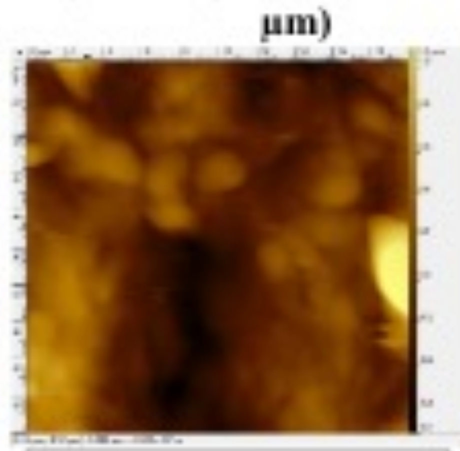
Fig 5. Image of Phase Scan (20 µm)



(18.42 µm,7.79 µ): -0.27µm = -2.667e-10 m

Fig 4. Image of Amplitude Error Scan

Fig 7 : Experimental setup employed for Nanoindentation



(3.14 µm,4.12 µm): 0.240 µm,= 2.402^-07 m

Fig 3: Image of Height Scan (20 µm)

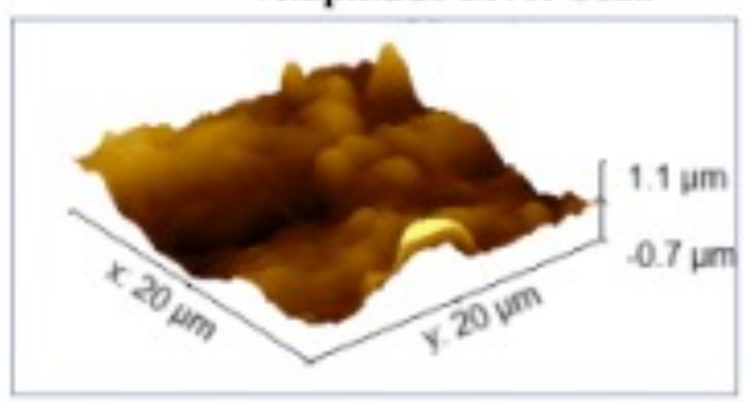


Fig 6. 3D Image of Height Scan (20 µm)

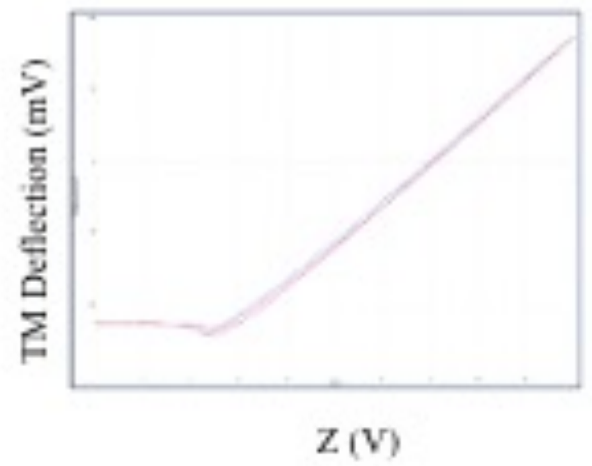


Fig 8. Multicurve Analysis from Nanscope- TM Deflection Sensitivity

Results

Young's Modulus (E) - Degummed SF	Young's Modulus (E) - Electrospun Silk fibroin (Literature)	Young's Modulus (E) - Electrospun Silk fibroin (Nanomechanical)
6-8 GPa	515 MPa	19.3 ± 2.8 GPa

Conclusion and Discussion

The objective of this project was to conduct nanomechanical tests on electrospun silk fibroin (SF) nanofibers using Atomic Force Microscopy (AFM) to accurately quantify load-strain behavior and comprehend their mechanical interaction with anchorage-dependent cells on electrospun substrates. This research is driven by the interest in advanced biomaterials for regenerative orthopedic products, aiming to enable the testing of nano stiffness with various fibers to tailor cell differentiation. Nanoindentation was employed via tapping mode of AFM to achieve this objective. The nanoindentation measurements were obtained using a DNISP probe with a radius of 40 nm. Young's modulus data were sourced from literature and supplemented with experimental results generated by members of the BioICAS laboratory. Post-processing of the height image involved a first-order plane fit, while observation of tilt was made via the 3D image, which may be affected by scanner bow, potentially introducing errors. Preliminary data obtained from nanoindentation provided insights into the system's behavior, particularly through TM deflection sensitivity. To further advance this study, the next steps involve reassessing results by measuring a single electrospun SF scaffold using a custom nanomechanical instrument. Additionally, conducting more AFM testing is essential to enhance the statistical validity of the findings. These endeavors are pivotal in advancing our understanding of nanomechanical properties in the context of electrospun SF nanofibers and their potential applications in regenerative orthopedics

References

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