Development of Fibronectin-Based Protein Fibers for Promoting Cell Adhesion to Biomaterial Textiles

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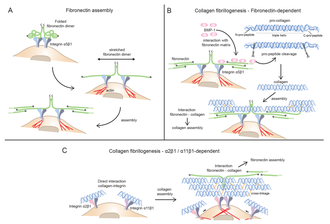
Introduction

- Tissue engineering combines cells with non-living materials and biologically active molecules to develop functional tissue constructs (Khademhosseini et al. 2009) which can be applied in drug development, disease modeling, and regenerative medicine.



- As the foundation of the extracellular matrix (ECM) and a crucial component of tissue assembly, these constructs are typically made with collagen-based scaffolds.
- By developing scaffolds which accurately mimic the structure of the ECM, cells can more easily reconstruct the internal structure of various tissues.

- Fibronectin (FN) is a promising candidate for protein fiber manufacturing and creating fibronectin-based biomaterial textiles for tissue engineering.
- Similarly to collagen FN, assembles into high order structures such as fibers, and plays critical roles in cell development, adhesion, and ECM development (Pankov and Yamada 2002).

- This study investigates the use of FN to produce stable protein fibers, by identifying a viable protein/synthetic polymer formulation using FN and Polyethylene(oxide)(PEO) and a single-pin contact drawing method.

Methods

Each protein/synthetic polymer formulation is tested through a dry analysis and hydration testing process.

- Initially, the dry fibers are created through the single-pin contact drawing method which extends the protein solution into a liquid filament suspended between two ends, which then dries to form the fiber.

This is done at room temperature, 21°C on average, and relative humidity ≤30%. Before undergoing hydration testing by a step-wise hydration process using PBS/PEO solutions, the fibers are exposed to at least one UVC radiation cycle at 200 mJ/cm2.

Hydration of the FN-PEO fiber

- Dissolves and removes the PEO
- Assesses the stability of the fibers

Step-wise hydration solutions

- 0.25PEO in 10x PBS
- 0.25PEO in 1x PBS
- 10xPBS
- 1x PBS

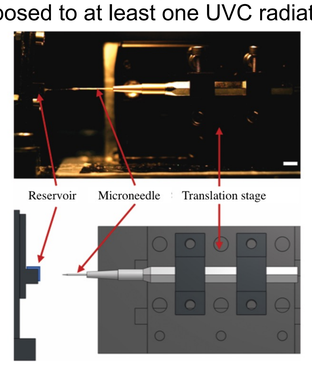


Figure 1. Single pin contact drawing setup for fiber fabrication. Displaying reservoir for placing protein-solution, 0.1mm needle, and translation stage. Image from Chowdhry et al. (2020).

Results

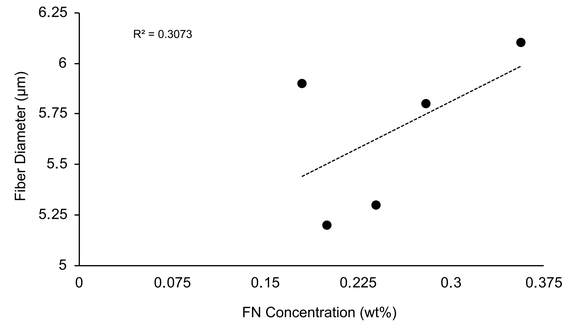


Figure 1. Average fiber diameter as a function of FN concentration in a solution using 1 wt% PEO. No strong correlation reflected ($R^2 < 4$), data points correspond to the average of at least 3 measurements.

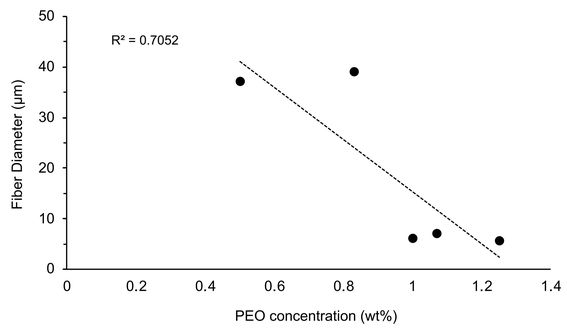


Figure 2. Average fiber diameter decreases as a function of PEO concentration (wt%) relative to FN in a solution using 0.36% FN.

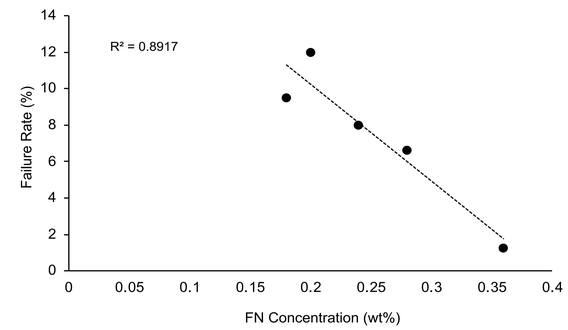


Figure 3. Average failure rate during single pin contact drawing cycles as a function of FN concentration, using 1% PEO, decreases linearly with increasing FN concentration (wt%). Modes of failure include fiber being released from one end before pull cycle was complete, and a liquid droplet developing along the fiber causing it to drop.

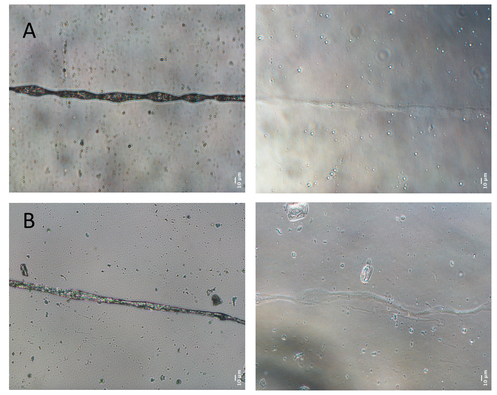


Figure 4. Single fibers before and after hydration. A) 0.36% FN-1wt% PEO fiber in 1xPBS solution. B) 0.2% FN-1wt% PEO fiber in 1xPBS solution.

Discussion and Conclusions

- Fibronectin FN can be used to manufacture protein fibers using a single-pin contact drawing method and an entangled polymer solution of FN and Polyethylene(oxide) (PEO).
- Due to the limited quantity of data at this time, a strong correlation can't be seen between fiber diameter and the concentrations of both FN and PEO.
- It becomes easier to pull fibers by single-pin contact drawing method when using increasing concentrations of FN.
- A lower concentration of FN (0.2%) supports more morphological consistencies in the fiber while also surviving hydration protocols.

Future Work

- Utilizing a heparin treatment to aid in FN fiber formation and stability (Hubbard et al.), further promoting the assembly of FN fibrils.
- Characterizing the mechanical properties of the fibers.
- At this stage, a multi-pin tool will be used to create non-woven textiles or scaffolds for cell culture testing.

References

[1] Hubbard B, Buczek-Thomas JA, Nugent MA, Smith ML. 2014. Heparin-dependent regulation of fibronectin matrix conformation. Matrix Biology. 34:124-131. https://doi.org/10.1016/j.matbio.2013.10.006
[2] Khademhosseini A, Vacanti JP, Langer R. 2009. Progress in tissue engineering. Scientific American. 300(5):64–71. http://www.jstor.org/stable/26001345.
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