Evaluating the Viability of Silk Fibroin as an Electrospun Biomimetic Extracellular Matrix

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Introduction

Electrospinning is a process in which nano-scale, fibrous synthetic polymeric scaffolds can be manufactured to mimic nature's scaffold, i.e., the extracellular matrix (ECM) by slowly extruding a polymer solution from a syringe across an electric field (Figure 1). The ECM serves as the framework that all anchorage dependent cells attach themselves to create 3D tissue and organs.

Specific aims for this project:

1. Develop a consistent manner and environment to successfully electrospin using a pullulan-dextran nano-crosslinked polymer solution
2. Assess the viability of silk fibroin (SF) as a natural biopolymer

Methods

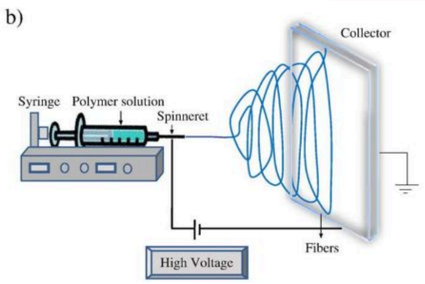
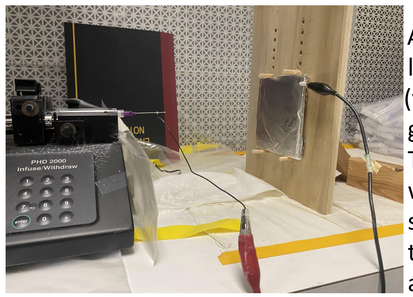


Figure 1. The Electrospinning Process (Bhardwaj & Kundu, 2010)

By applying a high voltage (15-25 kV) over a short distance (10-20 cm) an electric field is made that will accelerate the media in the syringe as it is slowly dispensed (0.8 - 3 mL/hr) to the point where as gravity is about to force the droplet to fall downwards, the force from the electric field becomes too powerful, forcing the droplet towards the target.



As shown in Figure 2, there is a large voltage applied to the needle (via the red wire), and the target is grounded (via the black wire). There is also a custom made stand with no metal components specifically designed to ensure that the droplets are not attracted to anything other than the target.

Figure 2. In lab electrospinning setup

By utilizing the easily manufactured pullulan-silk nano-crosslinked solution during the optimization of operating conditions, the harder to obtain SF is saved until the operating conditions are optimized.

Results

Optical Microscopy: By utilizing optical microscopy, a close up view of the scaffolding can be used to characterize the artificial ECMs.

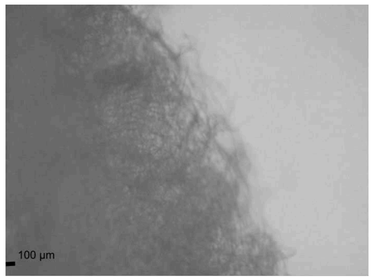


Figure 3. 100x magnification of pullulan-dextran electrospun scaffolding

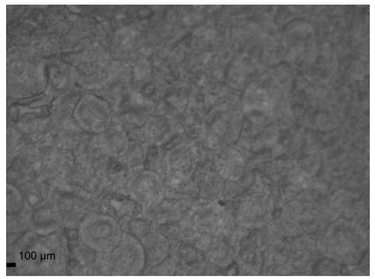


Figure 4. 100x magnification of SF electrospun scaffolding

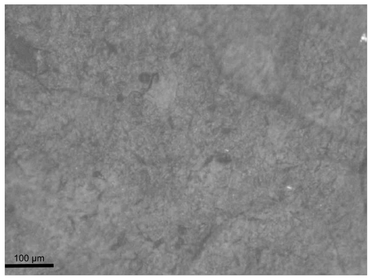


Figure 5. 500x magnification of SF electrospun scaffolding

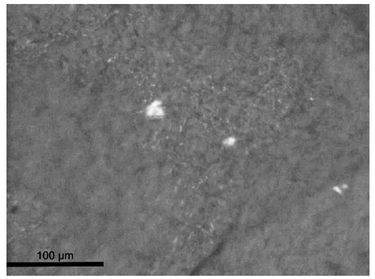


Figure 6. 1000x magnification of SF electrospun scaffolding

Discussion

As shown in Figure 3, the pullulan-dextran solution resulted in visibly separate fibers, which is ideal since these fibers provide a porous 3D architecture for anchorage dependent cells to anchor and proliferate. This is in stark contrast to the SF electrospun scaffolding, which has no discernible separate fibers up to 1000x magnification (Figures 4-6). This could be due to a number of reasons, from limitations of inhouse imaging equipment, as well as the very preliminary and non-optimized processing conditions. Due to limited supplies of SF, the solution used in the electrospinning process was made inhouse, and thus could be prone to a number of degumming processing issues. There were also 2 test variables that could not be controlled during electrospinning (surrounding temperature and humidity), although future measures will be taken to ensure these variables are under control.

Conclusions

As depicted in Figure 3, pullulan-dextran nano-crosslinked fibers were successfully spun. Electrospun SF solution did not produce fibers and will require further development. It is clear from the results that more optimization for the operating conditions in regards to SF is necessary. However, from literature reviews it is clear that once processing parameters are determined, electrospinning of SF fibers should be possible, repeatable, and most importantly have use in regenerative medicine.

Next Steps

Although SF was not successfully electrospun into a fibrous artificial ECM, steps have been already taken to run additional experiments in order to optimize the electrospinning conditions to facilitate fibrous results with SF. The utilization of optical microscopy will continued to be used during this stage in order to ensure that the SF scaffolding is not only fibrous, but free of beading. "Beading" is a phenomenon in the electrospinning process in which the polymer solution forms microscopic spherical masses of the media, also known as beads. Beading adversely affects the use of biomimetic ECM scaffolds - the more beading a scaffolding has, the less effective is its use for the development of 3D tissue. To combat beading, operating conditions such as surrounding temperature/humidity, media viscosity, voltage difference, and distance from the needle to target can be altered in order to minimize the formation of beads, resulting in more optimal biomimetic ECMs for 3D tissue engineering.

References

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