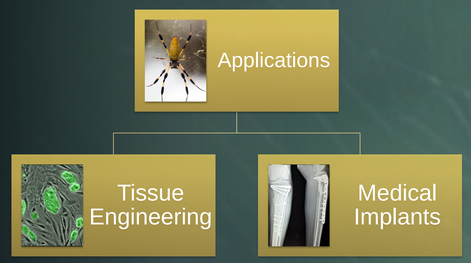
ARAKNIPRINT: 3D Printing of Synthetic Spider Silk to Produce Biocompatible and Resorbable Biomaterials

Ashley Ruben, Brianne Bell, Chase Spencer, Craig Soelberg, Dan Gil, Thomas Harris, Richard Decker, Timothy A. Taylor and Randolph V. Lewis

Utah State University, Department of Biological Engineering

Introduction

At $3.07 billion in 2013, the 3D printing industry was projected to reach $12.8 billion in 2018 and exceed $21 billion by 2020 [1]. A lucrative part of this expanding industry includes printing biocompatible medical implants, devices and tissue scaffolds. A common problem encountered with traditional devices and implants, is that they are not unique to the patient, making the surgeries more difficult and less effective. Tissue scaffolds could also benefit from increased strength and biocompatibility. To answer these demands, customizable devices are being produced from patient medical scans and CAD designs using 3D printers. Traditionally, plastics such as poly (lactic acid) (PLA) or poly (lactic-co-glycolic) acid (PLGA) are used in 3D printers because of their thermoplastic properties, which make them easy to print. These plastics are typically regarded as biocompatible but can degrade to less biocompatible forms in the body and leave the implant site, causing inflammatory and foreign body responses. Because of these problems, there has been a focus on developing new biomaterials for making customizable and highly biocompatible, resorbable implants.

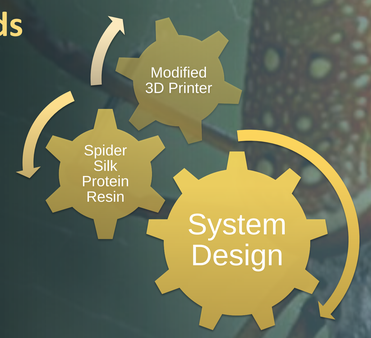


A 3D printer system comprised of a synthetic spider silk resin and a modified 3D printer was developed. A fused filament 3D printer, purchased for under $550, was modified with a custom syringe pump design. This syringe pump allowed for the extrusion of spider silk proteins through a needle, producing defined structures. Cell studies were performed on these structures which showed favorable cell attachment and growth. As an alternative 3D printed biomaterial, spider silk is capable of entering various emerging industries.

Design Objectives

1. Produce a defined structure from spider silk protein using a 3D printer.
2. Maintain inherent biocompatibility of synthetic spider silk proteins.
3. Achieve comparable mechanical properties to similar implants used in medicine.

Methods



Resin Formulation

Two spider silk protein resins were developed from rMaSp1 and rMaSp1 recombinant spider silk proteins derived from goats. The first was 6% by weight in a 50:50 ratio and the second was a 12% rMaSp1 only.

Printer Setup

The modified Printrbot Simple Metal, as shown in Figure 1a, uses Cura software. The printer was modified with a 3D printed syringe pump utilizing a 5 ml syringe and a 0.7 mm diameter needle (see Figure 1b).

Biocompatibility Test

This was performed using an alamarBlue™ assay. Thin resin films in 6 well plates were seeded with 300,000 goat fetal fibroblasts per well. 200ul of alamarBlue™ was added to each well and allowed to permeate the cells for 6 hours before absorbance readings were taken at 570 nm and 600 nm in a UV/VIS spectrophotometer.

Mechanical Testing

Simple structures were post-treated by soaking in either IPA, IPA & Cross-linking Solution (XLS), or XLS only for 24 hours. These were tested in shear and to the bio-yield point in compression on a 50 N load cell.

Results

Table 1. Final Results

Characteristic	
Printer Design	
Total Cost	$635
*Cost of Commercial Lab 3D Printer	$100-150 K
Aspect Ratio	4.8
Number of Spider Silk Protein Resins	2
AlamarBlue™ Assay: Cell Growth Inhibition †	
12% rMaSp1 Cross-linked	29.3%
6% rMaSp1:rMaSp2 IPA Post-treated	72.9%
6% rMaSp1:rMaSp2	42.2%
Mechanical Data	
Maximum Stress – Compression	65.1 ± 12.2 kPa
Energy to Break	10.7 ± 2.3 kPa
Elastic Modulus	555.8 ± 215.1 kPa
*Elastic Modulus 3D Printed *B. Mori* Silk	~0.2 kPa
Maximum Strain	0.247 ± 0.037 kPa
Maximum Stress – Shear	522 ± 403 kPa
*PLGA – Shear	~100 MPa

Data are presented as mean ± standard deviation
*Values from literature
†Values are percentages with respect to the controls

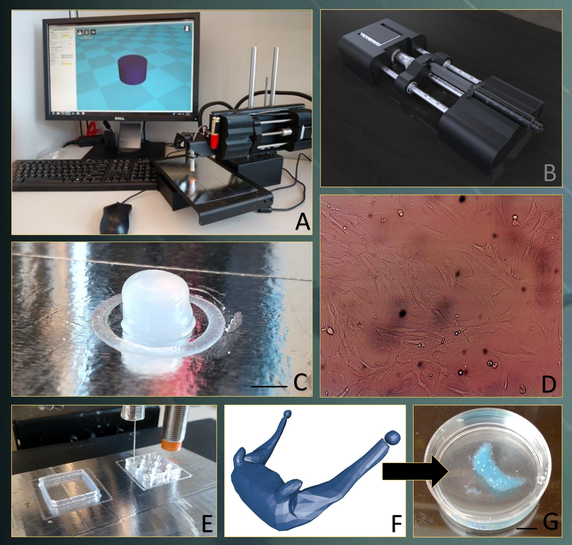


Figure 1. A) Completed system with modified syringe pump attached to Printrbot Simple Metal 3D printer, B) CAD rendering of syringe pump mechanism, C) Printed 5mm x 8mm spider silk cylinder used in mechanical testing (4mm scale bar), D) Goat fetal fibroblast cells showing favorable growth on cured spider silk resin, E) 3D printed spider silk showing aspect ratio, F) Scanned model of human hyoid bone (NIH 3D Print Exchange), G) 3D printed spider silk hyoid bone dyed blue and scaled down by ¼ (7mm scale bar).

Discussion

3D Printer Design

The total cost of the designed 3D printer was significantly less than commercially available aqueous 3D printers (see Table 1). The silk was also able to be printed at an aspect ratio of 4.8 (see Table 1, Figure 1e)

Biocompatibility

It was found that all resins inhibited cell growth with the lowest inhibition being 29.3% when compared to the controls (see Table 1). However, favorable cell attachment was shown (see Figure 1d).

Mechanical Data

The printed spider silk had a greater Elastic Modulus than those reported in literature for 3D printed *B. mori* silk. However, the spider silk had significantly lower shear values than those reported for PLGA (see Table 1).

Conclusion

Therefore the project was successful in designing an inexpensive 3D printing system comprised of a spider silk protein resin and modified FDM 3D printer that produced defined spider silk structures. An aspect ratio of 4.8, 29.3% cell inhibition, and compressive stress of 65.1± 12.2 kPa were achieved. The system provides a reliable platform on which to continue research.

Future Work

With the printing system in place, further studies will be conducted to test the silk's ability to perform as a medical implant material and tissue scaffold. Additional medical scans of increasing complexity will also be printed to test the applicable resolution of the printer, similar to the hyoid bone print seen in Figure 1f-g.

References

1. Wohlers, T., and T. Caffrey, 2013, Additive manufacturing and 3D printing state of the industry annual worldwide progress report. 2014: Wohlers Associates.

Acknowledgement

Study conducted with funding from a USU Undergraduate Research and Creative Opportunity Grant. Special thanks to Dr. Randolph Lewis, Dr. Justin Jones, Dr. Timothy Taylor, Richard Decker, Dan Gil and Thomas Harris for their advice and contributions.