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

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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.

Design Objectives
1. Produce a defined structure from spider silk protein using a 3D printer.
3. Achieve comparable mechanical properties to similar implants used in medicine.

Methods

Tissue Engineering
Medical 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.

Results

Table 1. Final Results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printer Design</td>
<td></td>
</tr>
<tr>
<td>Total Cost</td>
<td>$635</td>
</tr>
<tr>
<td>*Cost of Commercial Lab 3D Printer</td>
<td>$100-150 K</td>
</tr>
<tr>
<td>Aspect Ratio</td>
<td>4.8</td>
</tr>
<tr>
<td>Number of Spider Silk Protein Resins</td>
<td>2</td>
</tr>
</tbody>
</table>

AlamarBlue™ Assay: Cell Growth Inhibition

1% 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: 555.0 ± 215.1 kPa
- *Elastic Modulus 3D Printed B. Mori Silk: -0.2 kPa
- Maximum Strain: 0.347 ± 0.037 kPa
- Maximum Stress – Shear: 522 ± 403 kPa
- *PLGA – Shear: ~100 kPa

Data are presented as mean ± standard deviation

*Values from literature
*Values are percentages with respect to the controls

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


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.

Applications

Medical Implants

System Design

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 Curio 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).

Biacompatibility 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.

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