Effect of Processing Temperature on Poly(lactic-co-glycolic acid) Scaffold Properties and Bioactivity of Insulin-like Growth Factor

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INTRODUCTION

Polymeric materials are commonly used to develop biodegradable scaffolds because they can be tailored to meet specific degradation rates, mechanical properties or drug release rates. Biodegradable polymeric scaffolds offer an ideal scenario by providing the initial mechanical support needed at the site of implantation and then degrading away to avoid any additional retrieval surgeries required to remove any foreign materials from the body. While the scaffold degrades the tissue regrows, creating a cell environment that promotes proliferation and differentiation[1]. Making the scaffold porous further promotes tissue growth by allowing cell infiltration. By incorporating a drug or growth factor, the scaffold can be further tailored to meet specific needs, such as stimulating formation of bone or cartilage, making it applicable to a wide range of uses.

BACKGROUND

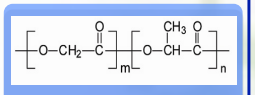

Figure 1. Structure of PLGA

Poly(lactic-co-glycolic acid) (PLGA) exhibits biodegradability and biocompatibility[2] with varying degradation, drug release and mechanical properties depending on the molecular weight. PLGA also allows for the encapsulation of growth factors, such as insulin-like growth factor I (IGF-I). IGF-I has been shown to stimulate the synthesis of proteoglycan and type-II collagen and enhances chondrocyte production of matrix synthesis[3]. The most common from of PLGA is microspheres, but lack the necessary mechanical properties to be suitable for a bone or cartilage implant. However, the proposed method of fabricating a scaffold by fusing together microspheres through incubation at or above the glass transition temperature (T_g), increasing the mechanical properties.

RESEARCH OBJECTIVE

To determine if the processing temperature needed to fuse the PLGA scaffolds, in order to obtain a higher compressive modulus, will adversely affect the bioactivity of IGF-I after its encapsulation.

MICROSPHERE FABRICATION
$W_1/O/W_2$ DOUBLE EMULSION TECHNIQUE

SCAFFOLD FABRICATION

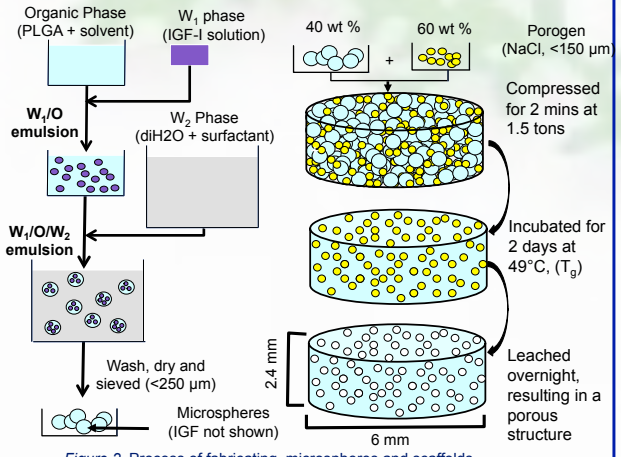

Figure 2. Process of fabricating microspheres and scaffolds.

MECHANICAL PROPERTIES

Incubation Temperature	Compressive Modulus (MPa) Dry (initial)	5 Days Degraded
49°C (at T_g)	111.8± 14.3	6.1±1.2
43°C (Below T_g)	10.2± 3.5	0.1±0.1

Chart 1. An increase of over 10X can be seen in the compressive modulus by incubating the scaffolds at the T_g.

IGF-I BIOACTIVITY ANALYSIS

Three step analysis:

Step 1
Incubated IGF-I solution at various temperatures and test bioactivity to strictly evaluate the effect of elevated temperatures

Step 2
Fabrication IGF-I scaffolds and determine release profile and degradation rate
Use supernatant to test bioactivity at various time points

Step 3
Seed cells directly onto scaffold to test cell response

IGF-I BIOACTIVITY- STEP 1
IGF-I SOLUTION INCUBATION

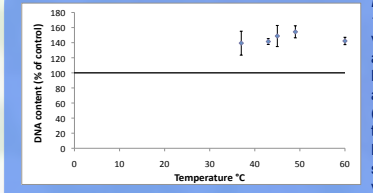

Figure 3. An IGF-I solution of 12 ng/mL was incubated at various temperatures and added to seeded cells. The DNA content was assayed and compared to the control (a blank solution incubated at the respected temperatures). Elevated levels of DNA were seen at each temperature, varifying the IGF-I bioactivity remains.

IGF-I BIOACTIVITY- STEP 2
IGF-I DRUG RELEASE AND SUPERNATANT BIOACTIVITY

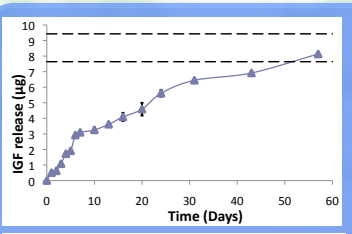

Figure 4. IGF-I release profile. The dashed lines represent the expected amount of IGF-I release based on the range of IGF encapsulated into the scaffold.
The release shows a small initial burst followed by a more linear release.

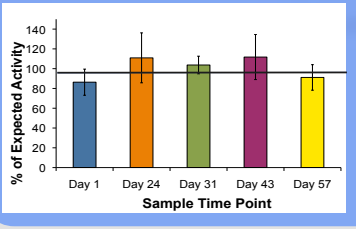

Figure 5. Supernatant was added onto seeded cells, assayed for DNA content and compared to the control (no IGF-I). Throughout the degradation process, which has been know to cause acidic buildup, no IGF-I activity was lost.

IGF BIOACTIVITY- STEP 3
CELLS SEEDED ONTO SCAFFOLD

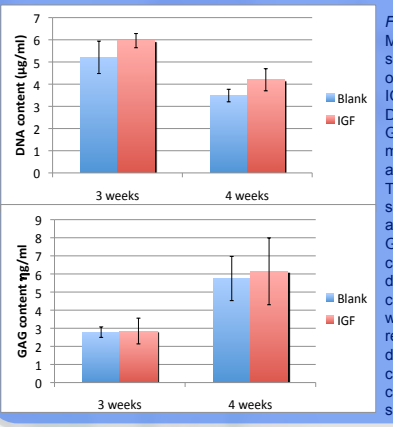

Figure 6. Bone Marrow Cells were seeded directly onto blank and IGF-I scaffolds. DNA content and GAG content were measured at 3 and 4 weeks. The IGF-I loaded scaffolds showed a slight increase in GAG and DNA content. The decrease in DNA content at 4 weeks could be a result of scaffold degradation carrying away cells and scaffold saturation.

DISCUSSION

- As expected with a PLGA scaffold or any other bulk degrading polymer, there was initially a higher release rate of IGF-I followed by a sustained release (seen in Figure 4). The higher initial release rate can also be attributed to the porous nature of the scaffold and increased surface area releasing any residual IGF-I molecules attached on the surface.
- The bioactivity of the IGF-I was not compromised by using the higher fusion temperatures or through the fabrication process. The activity was statistically similar to that of unhandled, stock IGF-I.
- IGF-I added to scaffolds shows a possible increased production of DNA and GAG content.

CONCLUSION

- IGF-I can can be encapsulated into PLGA microspheres and fabricated into scaffolds that use elevated temperatures without compromising the bioactivity. Using the heat sintering process allows for the fusion of microspheres leading to increased mechanical properties compared to non-fused scaffolds making it applicable to a wider range of of implant sites, including bone and cartilage.

REFERENCES

1) Advanced Drug Delivery Reviews, 2003. 55(4). 501-518.
2) Advanced Drug Delivery. 1997. 28. 5-24.
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ACKNOWLEDGEMENTS

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