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

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Introduction: Poly(lactic-co-glycolic acid) (PLGA) is commonly used as a scaffold because it exhibits biodegradable and biocompatible properties. Its properties can be tailored easily, such as by altering the molecular weight, allowing for finite control over the degradation, drug release, and mechanical properties. Furthermore, PLGA allows for the encapsulation of growth factors, such as insulin-like growth factor I (IGF-I), which has been shown to stimulate the synthesis of proteoglycan and type-II collagen while enhancing chondrocyte matrix synthesis (J Orthop Res, 1999, 467-74). The objective is to determine whether or not there is an effect of temperature on IGF-I after being encapsulated in a PGLA scaffold and it's ability to increase cell proliferation.

Methods: Two types of PLGA (50:50, acid-terminated) microspheres, uniform and blended, were prepared using the $W_1/O/W_2$ double emulsion technique. The uniform microspheres had a molecular weight of either 6 kDa, referred to as low molecular weight (LMW), or 30 kDa noted as high molecular weight (HMW). The blended microspheres contained polymers of both molecular weights at a 50:50 ratio and mixed during the first emulsion. The microspheres were sieved and mixed with 60 wt% of uniformly sized NaCl (<150 μm), which acts as a porogen. After consolidation using a Carver press and "sintering" at a controlled temperature, described later, salt was leached in deionized water. Mixed scaffolds were made by mixing LMW and HMW microspheres at various ratios before consolidation. The total mass of microspheres and NaCl per disk was constant. The sintering temperature used was based on the glass transition temperature (T_g), found to be 43°C, 45°C and 49°C for LMW, blended and HMW microspheres. The final scaffolds had a mass of approximately 44 mg and diameter of 6.0 mm.

To study the effect of processing temperatures on growth factor activity, an IGF-I solution at a concentration of 12 μg/mL in deionize water was incubated at 37, 43, 45, 49, or 60°C for 2 days. The bioactivity of the IGF-I after heat treatment was determined using a proliferation assay. SaOS human osteosarcoma cells were seeded into a 24-well plate and allowed to settle overnight. The medium was removed and McCoy's medium and the IGF-I solutions were added to each well. After four days of incubation at 37 °C, cell proliferation was assessed by measuring DNA contents with a Hoechst assay.

Results and Discussion: Compression tests showed that there was a significant difference, up to a 10-fold increase in strength, by increasing the temperature up to or above the T_g (Figure 1). This mechanical property is important for maintaining the microarchitecture for cell ingrowth and matrix synthesis without collapsing. However, using a higher temperature could compromise the bioactivity of the encapsulated protein.

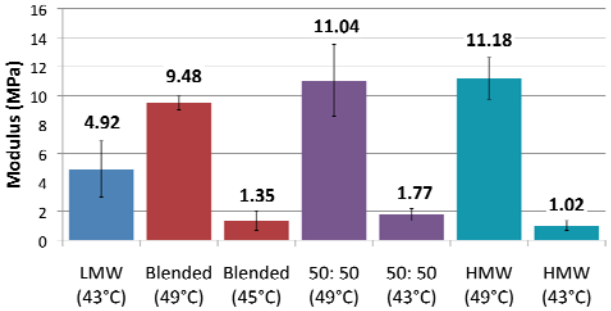

Fig 1. Compressive modulus at various temperatures.

Figure 2 shows the temperature effects on an IGF-I solution. The range of temperatures was chosen based on the temperatures necessary for fabricating the scaffolds. These results suggest that the elevated temperatures did not adversely affect bioactivity of IGF-I based on the statistically (p<.001) similar DNA contents compared to normal 37ºC incubation; IGF-I stimulated about a 140% increase in proliferation compared to cultures without growth factor.

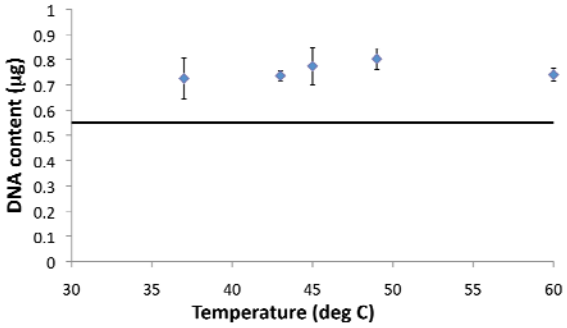

Fig 2. DNA content of cell cultures treated with IGF-I solutions incubated at increasing temperatrues. The horizontal line represents the control, where no IGF-I was added.

Conclusion: Higher compressive strength of the scaffold is necessary to withstand physiologic conditions, requiring the use of higher temperatures during fabrication. These elevated temperatures, however, did not affect the bioactivity of the IGF-I solution.

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