ENGINEERING A MULTIFUNCTIONAL SCAFFOLD FOR SPINAL CORD REPAIR

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INTRODUCTION

Spinal cord injury (SCI) is a devastating condition affecting nearly a quarter of a million Americans [1]. The majority of SCI victims are young adults who are often left with life long injuries including paralysis, respiratory, bowel and bladder dysfunctions. What makes these injuries so devastating is inability of the central nervous system (CNS) to regenerate the severed axons or blood vessels. Current treatment options for SCI focus mainly on relieving the compression on the spinal column and treatment of the symptoms.

Reference [2] was the first to show that regeneration of axons is possible with external stimulation. Further study into neurobiology has shown that the most promising stimulants for axon regeneration are neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial-cell line-derived neurotrophic factor (GDNF) [3,4,5]. Researchers have also shown that it is possible to block certain growth inhibitory responses that the body up-regulates post SCI [6]. While these trophic factors show great potential, the delivery of these highly lipophilic drugs to the CNS is difficult because they cannot pass the blood-brain-barrier (BBB), a highly selective membrane that protects the CNS. Direct injection of the trophic factors can flood the system, which is toxic to the local tissue [7]. A controlled sustained release of these trophic factors would lead to the highest efficacy and can be obtained by incorporation into degradable microparticles.

Along with the incorporation of neurotrophic factors, axonal regeneration also requires angiogenesis (the generation of blood vessels)[8]. Vascular endothelial growth factor (VEGF) is known to promote angiogenesis [9] as well as a stable, protected environment.

The novel solution to SCI is to combine these approaches and provide not only a mechanical stabilizer for the injured area of the spinal column, but also a localized release of therapeutic proteins. The scaffold proposed is a hydrogel of poly (2-hyrdroxy-ethylmethacrylate) grafted with poly (ethylene glycol). This scaffold could be adapted to match the mechanical properties (mainly the compressive modulus) of the local gray matter in the spinal cord (3-5kPa) [10]. The scaffold would have incorporated degradable microparticles. As these particles degrade, the entrapped therapeutic proteins would be locally released into the gray matter. The scaffold would also provide a protected environment in which blood vessels as well as axons could regenerate.

EXPERIMENTAL METHODS

Microparticles were made using a double emulsion- solvent-extraction method (W/O/W). The primary emulsion (PLA in solvent and model proteins plus HSA in citrate buffer) was mixed via sonication, then added to 5 (w/v)% PVA for the second emulsion and mixed at 400RPM overnight. Particles were collected via centrifugation, washed with dH_20 and lyophilized. The degradable polymer shell was made from poly (lactic acid) (PLA). The scaffold was made using a free radical co-polymerization of HEMA and PEG (200). Redox imitations were chosen so that the temperature would remain at physiological conditions in order to protect the stability of the therapeutic proteins. Gelation was created using TEGDMA as a cross-linker and controlling the solvent to polymer ratio.

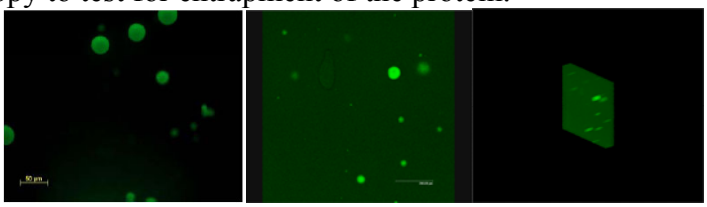
Protein-loaded particles were incorporated into the hydrogel through the solvent phase. Changing the solvent phase to a 50/50 mixture of glycerol/water allowed for resuspension of the particles. Polymerization with the particles dispersed in the solvent lead to entrapped particles in the hydrogel. The

compressive modulus of the scaffolds was tested using an Instron Series IX. The compression tests were done at a constant strain rate, in dH_20 held at 37^0C. The compressive modulus was found using the linear region of the stress-strain curve, which was found to be from 0-15% strain.

Release studies were done on 2mL samples of both hydrogels loaded with varying concentration of particles as well as particles alone. Release studies were performed in sink conditions in a Dissolution cell. Samples were taken at designated times and quantified through a combination of UV absorbance and reverse-phase HPLC.

RESULTS AND DISCUSSION

Particle synthesis was tested using FITC-ovalbumin loaded PLA particles. The FITC could then be seen under fluorescent microscopy to test for entrapment of the protein.



(a) (b) (c)

Figure 1. (a)Fluorescent image of FITC-ovalbumin loaded PLA-microparticles. Scale bar is 50µm. (b) Confocal microscopy of these particles in a PHEMA scaffold. Scale bar is 200µm. (c) Image (b) rotated to side view.

The image shown in Fig. 1 shows that the fluorescent protein was successfully entrapped into the particles and the particles are on the micron scale. Particles were also viewed using ESEM and found to be on the same size scale. Using a combination of ESEM images and confocal microscopy, the dispersion of the particles in the hydrogel could be tested. Fig.1(b &c) shows a slice from FITC-ovalbumin–PLA-particles loaded in a PHEMA scaffold. These images show that the particles are incorporated into the hydrogel matrix and not merely on the surfaces.

Control of the compressive modulus of the scaffold is a critical design parameter. The ideal is to match the compressive modulus of the gray matter in the spinal cord (3-5kPa). Preliminary results have shown that varying the amount of solvent phase (water) during the free-radical polymerization of the PHEMA scaffolds drastically changes the compressive modulus. The amount of particles added to the scaffold also greatly affects the resulting compressive modulus of the scaffold. These results are summarized in Figure 2 Figure 2 shows the result of varying the percentage of solvent during polymerization as well as increasing the weight percent of particles incorporated. It is clear that increasing the amount of solvent decreases the compressive modulus, and the ideal range is between 70% and 75% solvent (water). The red line on the bar graph is to indicate the target value. As for the increasing weight percentage of particles present, there is no clear trend as to their effect, but it is clear they do change the material properties.

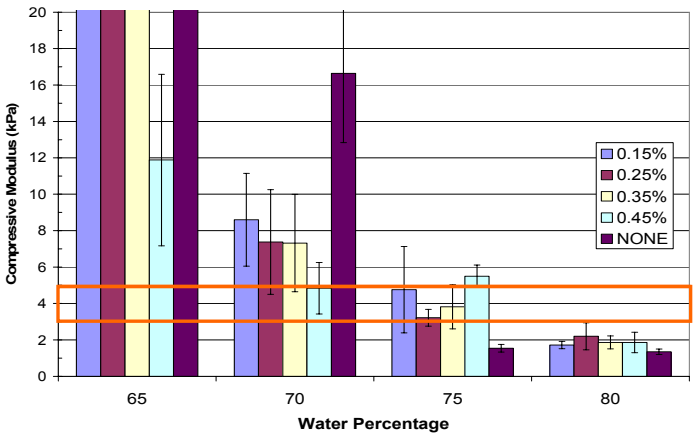


Figure 2. Compressive modulus testing for varying amount of solvent(water) present during polymerization with microparticles present in the solvent phase.

Release studies were conducted using drug-loaded PLA particles in a 70% solvent PHEMA scaffold at two different weight percents of particle loading. Results from this release study (done in triplicate) were analyzed using UV absorbance and can be seen in Figure 3. Release was sustained for two weeks at a relatively constant level that was attained within the first four days.

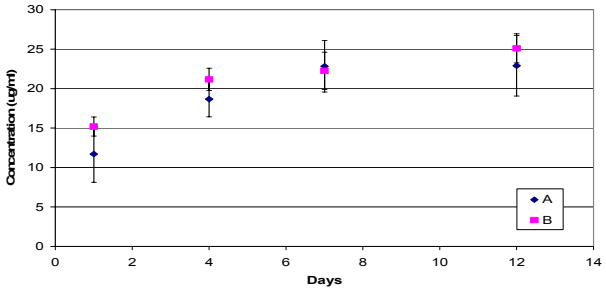


Figure 3. Release of albumin from PLA particles entrapped in 70% solvent PHEMA hydrogels at A(wt%) and B (wt%).

CONCLUSION

Preliminary results have shown that it is possible to entrap the model proteins into the PLA microparticles and that these particles can then be successfully dispersed into the hydrogel matrix. Mechanical studies have shown that the compressive modulus is highly adaptive to changes in solvent to polymer ratio, as well as the amount of particles present. These variables will allow for the fine-tuning of the material properties. Investigation of the stability of the protein during this fabrication process is being conducted.

Release studies thus far have shown that release can be sustained for up to two weeks from the scaffold. Future work will involve further mechanical and release studies as well looking into the biocompatibility of the scaffold and released drug as well as *in vivo* studies in adult rats.

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