Bioabsorbable Anti-inflammatory Material: A Novel Protein-Conjugated Polymer with Applications in the Implantable Medical Device Field

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ABSTRACT

In this paper, we present a microfluidic method of creating hybrid protein-PLGA particles. We are combining the bioabsorbable property of PLGA and the anti-inflammatory property of an immunomodulatory protein. We aim to assess whether this protein can inhibit inflammatory response to PLGA's acidic subunits as it degrades. Particles are made using a double emulsion and solvent extraction process. To date, our microfluidic system has been optimized to create single or multi-cavity particles, thin shell particles, and solid particles using a non-toxic solvent. In the near future we will characterize and compare the release profiles of the hybrid protein-PLGA particles vs. conventional protein encapsulated particles.

Keywords: PLGA, stent, particles, microfluidics, anti-inflammatory, immunomodulatory

INTRODUCTION

The human immune system is designed to recognize and fight "foreign" objects, pathogens, or domestic diseased cells. Part of the immune system's defenses includes an inflammatory response in which the area surrounding damaged or infected tissue becomes red and swollen (inflamed). This is in part due to white blood cell activation from the presence of a foreign object or laceration. Inflammation poses a major problem to the successful function of essentially "foreign" implanted medical devices such as vascular stents, heart valves, hip or knee replacements, etc. Conventional implants are permanent and made from surgical grade stainless steel metal which does not provoke an immune response. However, there is a need for non-permanent implant solutions. Biodegradable/bioabsorbable materials are a relatively new movement in the multibillion dollar implantable medical device field. These materials break down into smaller absorbable subunits in the body after it has served its purpose. Despite the promise of non-permanent implants, subunits of degraded bioabsorbable materials can induce a localized inflammatory immune response. This can be seen in a commonly used bioabsorbable material called Poly(lactic-co-glycolic acid) (PLGA), which is currently used as degradable stent and suture material among other things. PLGA degrades into nontoxic lactic and glycolic acid subunits. Although PLGA is a safe material for implantation into the body, accumulation of its acidic subunits in a localized area can cause irritation thereby invoking an inflammatory response. To overcome this limitation, one must design a degradable polymer that is capable of reducing acute inflammation while promoting wound healing. In previous studies, an immunomodulatory protein coated on polystyrene surfaces has been found to reduce the activation of white blood cells and their subsequent secretion of inflammatory factors [1]. Our work aims to combine the bioabsorbable property of PLGA and the anti-inflammatory property of an immunomodulatory protein described by [1]. We are using a microfluidic system designed to conjugate functionalized proteins to PLGA, forming protein-polymer hybrid particles. Our lab has previously reported on two microfluidic approaches for the synthesis of PLGA micro and nanoparticles using a non-toxic organic solvent [2]. We are assessing whether an immunomodulatory protein can be conjugated to PLGA microfluidically, displayed throughout the particle lifetime, and retain its functionality. For the purpose of this study, bovine serum albumin (BSA) is used as a model protein to test the feasibility of protein-polymer conjugation and to fine-tune the parameters of microfluidic particle production. The next step is to conjugate PLGA with an immunomodulatory protein as described by [1] and observe its functionality. In the future, we hope to create vascular stents with this anti-inflammatory PLGA material which would provide a non-permanent, biodegradable alternative to conventional stent materials.

APPROACH

Microfluidic devices are fabricated by the poly(dimethyl) siloxane (PDMS) replica molding process using standard soft lithography techniques [3]. Device

design includes two flow focusing junctions, the first junction (Fig. 1)creates discrete droplets of protein immersed in a PLGA-solvent solution (1-2.5wt% PLGA 50:50 or 2wt% PLGA 85:15 in DMC (dimethyl carbonate)). The second junction (Fig. 2) creates water/oil/water double or multi emulsions with 1-2.5wt% PVA (polyvinyl alcohol) solution as the continuous phase. The device outlet is connected to a mixing well where the emulsions (Fig. 3) are stirred to help the solvent evaporation process and the subsequent formation of particles (Fig. 4). Particles are formed after the non-toxic solvent, DMC, is evaporated from the emulsion while in aqueous solution. Particles are collected, centrifuged, and washed several times before further testing.

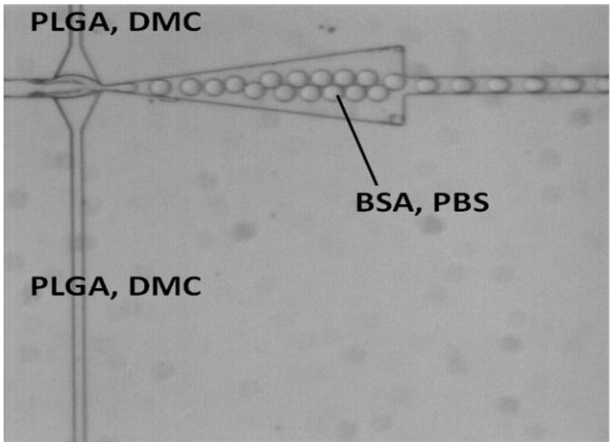

Figure 1: Microfluidic device, primary flow focusing junction.

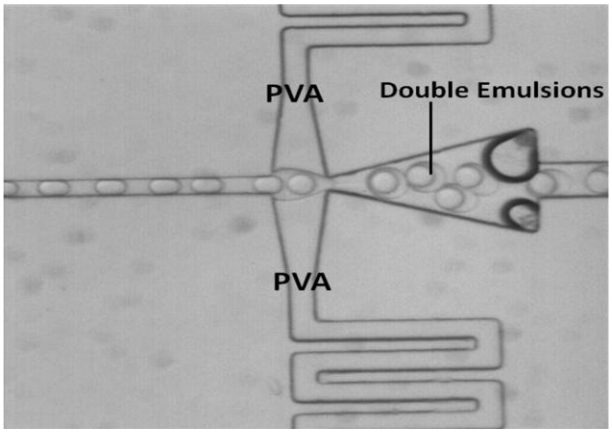

Figure 2: Microfluidic device, secondary flow focusing junction.

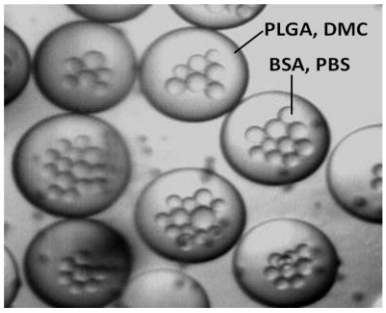

Figure 3: Multi-emulsion PLGA droplets before solvent evaporation (4X magnification).

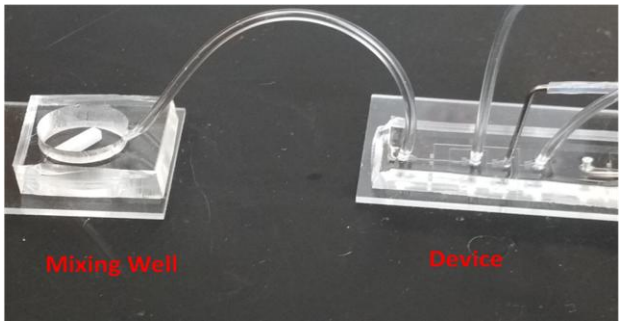

Figure 4: PDMS microfluidic device and PDMS mixing well

RESULTS & DISCUSSION

To date, our microfluidic system has been optimized to create single or multi-cavity particles, thin shell particles, and solid particles using a non-toxic solvent (**Fig. 5**). To monitor and characterize the release profiles of the particles, we have encapsulated fluorescent BSA (**Fig. 6**). We are currently comparing two approaches for the conjugation of protein to functionalized PLGA. In the near future we will characterize and compare the release profiles of the hybrid protein-PLGA particles vs. conventional protein encapsulated particles. Once the BSA release profiles are characterized, CD200 will be substituted in place of BSA. Particles will be created with different dosages of CD200, cultured with macrophage, and extensively observed for biological response to its degradation. This includes observing CD200-PLGA particle influence on cell activation and secretion of pro-inflammatory factors.

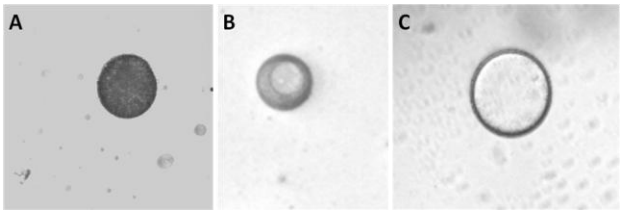

Figure 5: Inverted microscope image of solid PLGA particle at 20X magnification (A), Single cavity particle at 20X magnification (B), and a thin shell particle at 10X magnification (C).

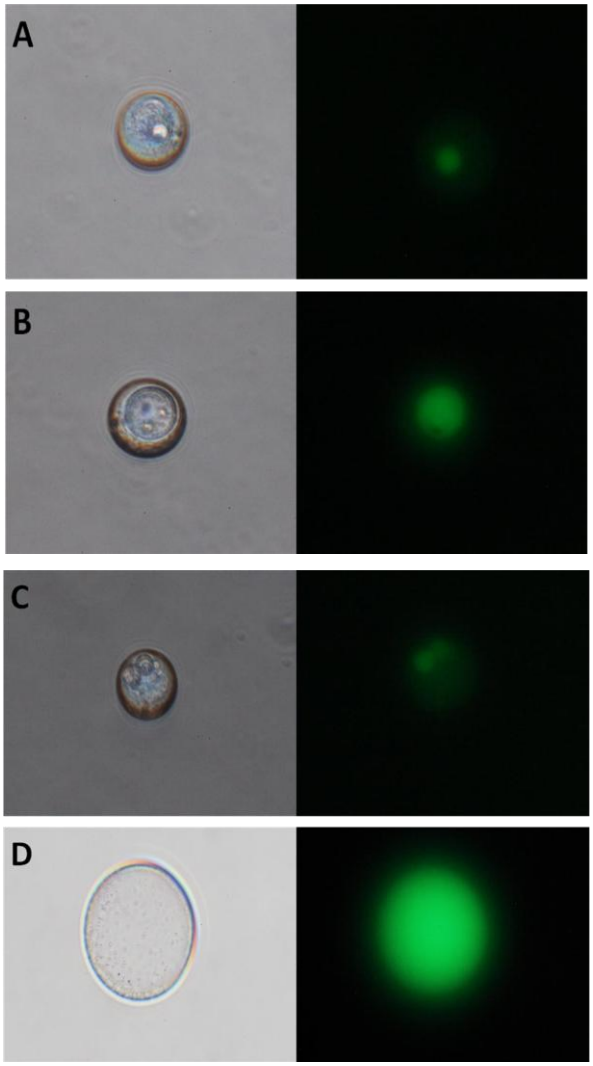

Figure 6: Inverted microscope bright field and FITC filtered images of PLGA particles encapsulating fluorescent BSA at 40X magnification. Images of a single small cavity particle (A), a large single cavity (B), a double cavity (C), and a large thin shell particle (D).

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