

Encapsulation and Characterization of Bovine Serum Albumin in PLA-PEG Nanoparticles

Murtada Taha, Shree R. Singh, Courtney Moore, Ronald Agee, and Vida A. Dennis*

Center for Nanobiotechnology and Life Science Research, Alabama State University, 1627 Hall St, Montgomery, AL 36104, vdennis@alasu.edu.

ABSTRACT

Chlamydia trachomatis is the most commonly reported bacterial sexually transmitted infectious agent worldwide. To date there is no efficacious vaccine against this human pathogen. PLA-PEG copolymer is one of the most promising and widely accepted delivery system for vaccines due to its biodegradable, biocompatible and bioresorbable nature. Thus the main goals of this study were to first successfully encapsulate and characterize a model protein namely, Bovine Serum Albumin (BSA) in PLA-PEG nanoparticles, and to similarly encapsulate and characterize a *C. trachomatis* recombinant protein (rMOMP-278) in these nanoparticles to serve as a nanovaccine delivery system against this pathogen. The carrier protein BSA was encapsulated in PLA-PEG by the double emulsion method and characterized using FTIR, which showed that the spectra of PLA-PEG-BSA corresponds to that of the PLA-PEG nanoparticles alone. The size and zeta potential measurements were found to be 94 nm and -12.17 mV respectively, which indicate it is very small in size and highly stable. The protein encapsulation efficiency of the PLA-PEG-BSA was found to be 34%, while the *in vitro* release study showed a slow and continuous release of the BSA protein from the PLA-PEG nanoparticles. The toxicity study of PLA-PEG to mouse J774 macrophages using the MTT assay revealed minimal toxicity up to 500 ug/ml.

Keywords: *C.trachomatis*, MOMP, PLA-PEG, BSA.

1 INTRODUCTION

Chlamydia trachomatis, a gram negative intracellular bacterium, is a major human health pathogen due to its role as the leading cause of bacterial sexually transmitted diseases in the world. There is no efficacious vaccine available against this human pathogen; therefore there is a need to develop

an effective vaccine at the mucosal surface against this pathogen. There were many attempts to develop a vaccine for *C. trachomatis* for several years; however, there was a lack of success. The major outer membrane protein (MOMP) is the most desirable proteins for use in the vaccine (1). However, purified MOMP and other subunits have been evaluated in several animal models with limited success, perhaps due to ineffective delivery systems or lack of effective adjuvants to boost mucosal immunity (2). Safe and effective delivery systems are required to protect and deliver rMOMP to antigen-presenting cells and to the genital mucosal inductive sites or the associated secondary lymphoid tissues (3). Poly (lactic acid)-poly (ethylene glycol) (PLA-PEG) copolymer is one of the most promising and widely accepted delivery system for vaccines due to its biodegradable, biocompatible and bioresorbable nature (4). Thus the main goals of this study were to first successfully encapsulate and characterize a model protein namely, Bovine Serum Albumin (BSA) in PLA-PEG nanoparticles, and to similarly encapsulate and characterize a *C. trachomatis* recombinant protein (rMOMP-278) in these nanoparticles to serve as a nanovaccine delivery system against this pathogen.

2 MATERIALS AND METHODS

2.1 Preparation of Nanoparticles

A w/o/w double emulsion-evaporation technique was used; 100 mg of the PLA-PEG was emulsified in 2 ml of dichloromethane. To the primary emulsion, 500 ug of BSA was added and homogenized using ultrasonic dismembrator (Fischer Scientific; model 150 E) at a continuous mode for 1 min at 20 sec interval on ice, followed by addition of 2 mL of PVA in distilled water (1%). The resulting double emulsion was transferred to 32.5 mL of PVA

for a total of 40 mL, gently stirred overnight at room temperature (RT) to evaporate the organic solvents resulting in formation of BSA encapsulated in PLA-PEG nanoparticles. Nanoparticles were harvested by centrifugation at 41,000 rpm, for 10 min., washed three times in distilled water and lyophilized for 10 hours using Labconco freeze dryer. Lyophilized nanoparticles were kept at -80 °C in a sealed container, until ready to be used.

2.2 Fourier Transform-Infrared (FT-IR)

Fourier Transform-Infrared (FT-IR) spectra were recorded for neat PLA-PEG-BSA and the PLA-PEG-PBS nanoparticles blends in attenuated total reflectance (ATR) mode using an IR spectrophotometer. The spectra were obtained with 64 scan per sample ranging from 4000 to 400 cm and a resolution of 4cm. The sample chamber was purged with dry N₂ gas.

2.3 PLA-PEG-BSA Nanoparticles Size and Zeta Potential Determinations

The particle size and zeta potential of PLA-PEG-BSA nanoparticles were measured by dynamic light scattering using Zetasizer Nano-ZS. Samples of PLA-PEG-BSA nanoparticles were suspended in distilled filtered water, sonicated, and then placed in a disposable cuvette for size and zeta potential measurements. Each sample was measured three times for triplicate preparations of nanoparticles and is reported as the mean.

2.4 *In vitro* Release of BSA from PLA-PEG Nanoparticles

The release of the BSA from the PLA-PEG was determined following the method of Bouissou et al (5). Briefly, PLA-PEG-BSA or PLA-PEG-PBS was suspended in 200 ul of 1 x PBS containing 0.01% sodium azide. The suspension was incubated horizontally at 37 °C and at various time intervals (30 minutes, 8, 24, 48, 72, 96, 120, 144, and 168 h) supernatants were collected by centrifugation at 12,000 rpm for 5 min and kept at -20 °C till used.

2.5 Cytotoxicity Assay

Cytotoxicity of PLA-PEG-BSA was measured using the MTT dye reduction assay in mouse J774 cells. Mouse J774 macrophages were seeded in 96-well plates at the density of 1.0×10^5 viable cells/well in 50 ul of complete media and incubated overnight at 37 °C under 5% CO₂ to allow the cells to attach. 50 ul of PLA-PEG was added to the cells in concentrations ranging from 7.8-1000 ug/ml. After 24 h, 15 ul of MTT dye solution were added into each well, and the cells were incubated for 4 hours at 37 °C under 5% CO₂. To stop the reaction, 100 ul of solubilization solution were added to each well and plates incubated for at least 1h at room temperature in the dark. Absorbance at 570 nm was measured using a microplate reader.

3 RESULTS AND DISCUSSIONS

3.1 Fourier Transform-Infrared (FT-IR) analysis

FT-IR was used to identify chemical functional groups in the nanoparticles, and used as an indication of successful encapsulation. The FTIR spectra of the PLA-PEG-PBS and PLA-PEG-BSA were showed in Fig. 1. A characteristic absorptive band at 1743.10, 1182.80, and at 1082.0 cm⁻¹ are due to the ester carbonyl (C=O), and C-O groups respectively. These spectra were found for both PLA-PEG-PBS (red) and PLA-PEG-BSA (blue) nanoparticles, which suggest the presence of the PLA-PEG in both nanoparticles, and successful encapsulation.

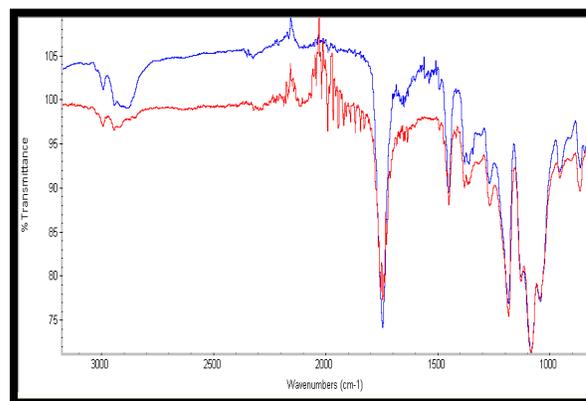


Figure 1: Infrared spectra of blank PLA-PEG-PBS and PLA-PEG-BSA nanoparticles.

3.2 Size Measurements

Particle size has been shown to directly affect the encapsulation efficiency and the release of the peptide from the nanoparticles. In this study, Zetasizer Nano techniques showed the mean particle size of PLA-PEG-BSA to correspond to 94 nm (Fig. 2). Formation of nano size particles is advantages for a nano-based vaccine such as *Chlamydia* vaccine, due to enhancing of the uptake of the nanoparticles by the immune cells, which will lead to better immune responses.

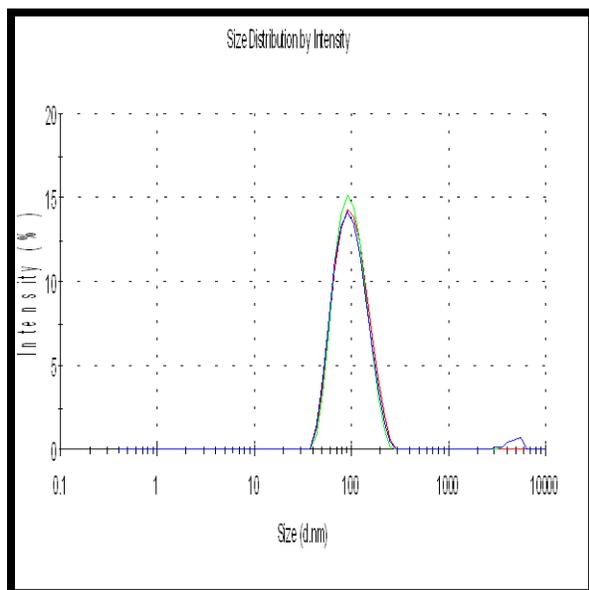


Figure 2: The mean particle size of PLA-PEG-BSA nanoparticles using Zetasizer Nano techniques.

3.3 Zeta Potential

Zeta potential of the nanoparticles can greatly influence their stability in suspension through electrostatic repulsion between the particles. High value of the zeta potential implies high surface charge of the nanoparticles, which leads to strong repulsive interactions between the nanoparticles and hence it's high stability (6). Figure 3 shows the zeta potential for PLA-PEG-BSA nanoparticles, which was found to be -12.17 mV. This absolute zeta potential value is high enough to allow us to suggest, the stability of PLA-PEG-BSA nanoparticles in the dispersion.

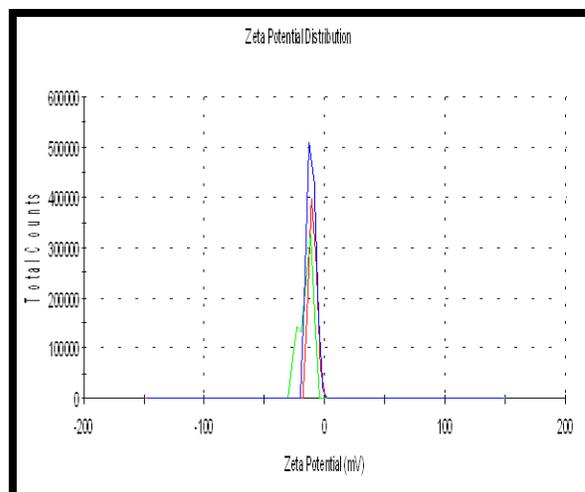


Figure 3: Zeta potential analyses of PLA-PEG-BSA using the Zetasizer Nano-ZS.

3.4 *In vitro* Release of the Peptide from the Nanoparticles

Figure 4 shows the release behavior of the BSA from PLA-PEG nanoparticles. The nanoparticles showed a biphasic pattern, characterized by a fast initial release up to 72 h, followed by a sustained slow release up to 168 h. This data demonstrated that the BSA released from the PLA-PEG nanoparticles increased with time, which imply that, this nanoparticles could be a good choice for encapsulating our rMOMP-278 peptide, as slow release is important for vaccine development

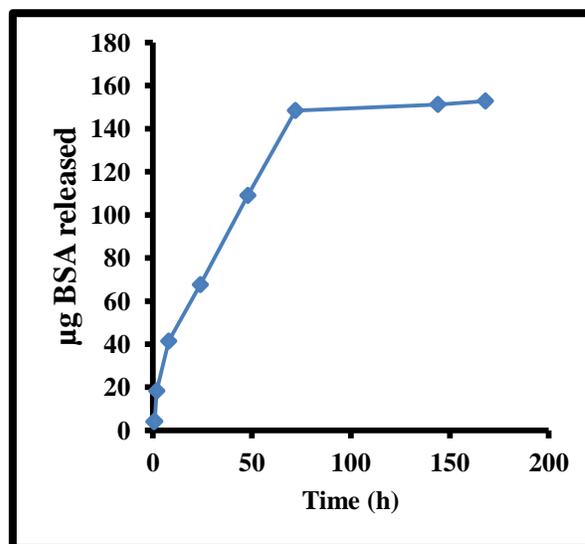


Figure 4: *In vitro* release of BSA from PLA-PEG nanoparticles.

3.5 Cytotoxicity Studies of PLA-PEG Nanoparticles

MTT assay was used to evaluate the cytotoxicity of PLA-PEG to mouse J774 macrophage. As shown in Fig. 5 the cell viability of J774 macrophages incubated with different concentrations of PLA-PEG for 24 h was above 80% for a dose as high as 1000 $\mu\text{g/mL}$. Overall, our cytotoxicity studies shown that, there were no adverse effects on cell viability by PLA-PEG, thus suggesting the safety of this delivery system for vaccine studies.

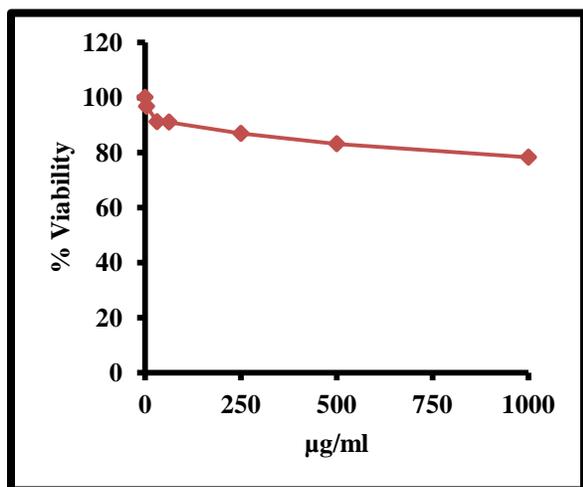


Figure 5: MTT assay for cytotoxicity of PLA-PEG-BSA nanoparticles to J774 macrophage cells after incubation for 24 h.

4 CONCLUSIONS

Overall, our study shows the successful encapsulation and characterization of our model BSA protein in PLA-PEG nanoparticles. Physicochemical characterization studies confirmed BSA encapsulation in PLA-PEG nanoparticles as small stable with slow release properties. Cytotoxicity studies indicate biosafety of PLA-PEG nanoparticles for *in vivo* studies as they exhibited little or no toxicity to mouse J774 macrophages. The rMOMP-278 protein encapsulated in PLA-PEG is currently being evaluated as a nanovaccine delivery system for *C. trachomatis*.

5 REFERENCES

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ACKNOWLEDGEMENTS

This project was supported by NSF-CREST grant HRD-0734232.