Influence of Polyvinyl Alcohol (PVA) on Morphology and Encapsulation Efficiency of Polylactic Acid-Polyethylene Glycol (PLA-PEG) copolymer based Nanoparticle's

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ABSTRACT

Biodegradable nanoparticles (NPs) have a wide range of possibilities and advantages when compared to other drug delivery systems. Biodegradable polymers typically employed for fabrication of NPs provide controlled release, low toxicity, high encapsulation efficiency, sub-cellular size and bio-distribution in vivo. They are also excellent for vaccines delivery and can be fabricated to evoke immune responses, either by direct stimulation of antigen presenting cells (APCs) or delivering antigens to specific cellular compartments. However, problems associated with NPs formulation include their homogeneity, and rapid protein release (burst effect). Much research has been conducted on the block copolymer polyethylene glycol and polylactic acid (PLA-PEG). Polyvinyl alcohol (PVA) is the most commonly used emulsifier in the formulation of NPs. PVA provides non-aggregating, emulsifying and adhesive properties to NPs and an interconnected network with the polymer at the interface which ultimately can affect their homogeneity, release profile and size[1]. The objective of this study was to determine the concentration-dependent effect of PVA on the size, morphology and release profiles of the modeled protein, bovine serum albumin (BSA) encapsulated within PLA-PEG. NPs were formulated by the Water/Oil/Water double emulsion method using concentrations of PVA ranging from 0.5-3%. Scanning electron microscopy (SEM) analyses revealed that use of 0.5 and 1.0 % PVA resulted in smaller NPs (126 - 226 nm). In contrast, 2 and 3% PVA resulted in larger NPs (160 - 312 nm). The encapsulation efficiency (EE) of NPs with 0.5, 2 and 3% PVA was 20-30%, while that of 1% PVA was 40%. Selection of 1% PVA for formulation of NPs with 2X polymer (200 mg PLA-PEG with 4 mg BSA) and less BSA (2 mg BSA and 200 mg PLA-PEG) resulted in 55% and 64% EE, respectively. These interesting observations will permit the selection of sizes of PLA-PEG NPs formulations for maximal interaction with APCs, but more importantly for vaccine delivery.

Key words: Polyvinyl alcohol (PVA) and poly (lactic acid)-b-Poly (ethylene glycol)

1.0 INTRODUCTION

PLA-PEG [poly (lactic acid)-poly (ethylene glycol), a biodegradable copolymer, has the ability to delay release of protein over several weeks, a characteristics that makes it attractive for vaccine delivery [2]. Encapsulation of biomaterials in PLA-PEG allows maintaining their integrity and activity, protecting them from exposure to extreme pH conditions, bile and pancreatic secretions, and augmenting their immune-potentiating effects. PLA-PEG sustained release, sub-cellular size, and enhanced biocompatibility properties facilitate uptake of antigens by APCs as well as increasing APCs influx to the injection site. PLA-PEG as a delivery vehicle for drugs is well documented as demonstrated by its capacity to enhance the activity of encapsulated drugs. However, there are limited studies exploiting the attractive properties of PLA-PEG for vaccine delivery and formulation of these nanoparticles for controlled release.

2.0 MATERIALS AND METHODS

2.1 Preparation of nanoparticles

BSA (bovine serum albumin), a model protein was encapsulated in PEG-b-PLA Diblock polymer nanoparticles by a modified water/oil/water double emulsion–evaporation technique essentially as described [3, 4]. Briefly 100 or 200 mg of PLA-PEG was emulsified in Ethyl acetate followed by addition of 2 or 4 mg of BSA , homogenization and than addition of 0.5-3% Polyvinyl Alcohol (PVA). The resulting double emulsion was gently stirred overnight at room temperature (RT) to evaporate the organic solvents, harvested by ultacentrifugation, washed and lyophilized in the presence of 5% trehalose. Sterile PBS was used in the primary emulsion formation to prepare PLA-PEG-PBS nanoparticles to serve as a negative control. Six different formulations of nanoparticles were made (**Table 1**).

2.2 Zeta potential and Zetasizer determinations

The size and zeta potential of NPs were measured by dynamic light scattering using a Zetasizer Nano-ZS

(Malvern Instruments, UK) as described [3, 4]. Samples of PLA-PEG-PBS and PLA-PEG-BSA were suspended in distilled water, sonicated for 5 min, and then placed in a

Table 1. **Different Formulations of Nanoparticles**

% PVA	Amount of polymer (mg)	Amount of (mg)
0.5	100	2
1	100	2
1 (2X PLA-PEG and BSA)	200	4
1 (2X PLA-PEG)	200	2
2	100	2
3	100	2

disposable cuvette for size and zeta potential measurements. Each sample was measured in triplicates for each preparation of NPs and is reported as the average reading.

2.3 Scanning electron microscopy (SEM)

The morphology of PLA-PEG-PBS and PLA-PEG-BSA was investigated using SEM (Zeiss EVO 50 VPSEM) as reported [3, 4]. The particles were mounted on metal pegs using conductive double-sided tape, and sputter coated with a gold layer prior to SEM analysis.

2.4 Encapsulation efficiency (EE)

The encapsulation efficiency was extrapolated from measurements of the total BSA encapsulated in PLA-PEG as described previously [3, 4]. Briefly, 20 mg of PLA-PEG-BSA was added to 1 mL of 0.1 N NaOH containing 2% SDS and shaken overnight at RT.
The supernatants were collected by centrifugation and stored at -20^{o}C until used. A Micro BCA protein assay was used to quantify BSA in supernatants, and absorbance was read at 562 nm using a microplate reader (TECAN US Inc., Durham, NC). Background readings were corrected by subtracting the optical density (OD) values of supernatants from PLA-PEG-PBS. The EE was calculated as: EE=A-B/A × 100 %, where A is the total BSA amount, B is the free BSA amount. These measurements were performed three times.

2.5 *In vitro* release and Ultra Violet Visualization (UV-Vis) Studies

The release of encapsulated BSA from PLA-PEG-BSA was determined as described [4]. Briefly, PLA-PEG-BSA and PLA-PEG-PBS (50 mg each) were each suspended in PBS containing 0.01% sodium azide. Suspensions were incubated at 37^{o}C and at various time intervals (1 h and up to day 10) supernatants were collected by centrifugation at 12,000 rpm for 5 min and kept at -20^{o}C until used. The Micro BCA protein assay was used to quantify BSA in supernatants and the absorbance was read at 562 nm using a microplate reader (TECAN US Inc).
Absorbance spectra of BSA and NPs were determined using the DU 800UV/Vis spectrophotometer program. NPs were diluted in 1 mL deionized water and the absorbance was measured to determine whether absorbtion of BSA occurred on the outside of NPs.

3.0 RESULTS AND DISCUSSIONS

3.1 Zeta potential and Zetasizer

Table 2. Zeta Potential of Nanoparticles

%PVA	**PLA-PEG-PBS(mV)**	**PLA-PEG-BSA(mV)**
0.5	-30.2	-50
1	-32.1	-50
2	-40	-40
3	-40	-40

The zeta potential value is one of the most important particle characteristics because it can affect both particle stability and particle muco-adhesion property. There was essentially no differences between zeta potential values before and after encapsulation in the 2 and 3% PVA formulations; in contrast, stablity is increased after encapsulation in the 0.5 and 1% PVA formulations **(Table 2)**. The average nanoparticle sizes obtained for 0.5, 1, 2 and 3% PVA formulations for PLA-PEG-PBS and PLA-PEG-BSA were 156, 123.2, 198.3 and 257.8 nm, respectively. These findings demonstrate that the encapsulation process had little effect on changing the properties and sizes of the NPs which are essential for maintaining their integrity.

3.2 Scanning electron microscopy (SEM)

SEM was employed to assess the morphological characteristics and sizes of encapsulated nanoparticles. The average sizes of PLA-PEG-PBS and PLA-PEG-BSA are within agreement with the zetasizer results.
SEM analyses revealed that use of 0.5 and 1.0% PVA resulted in similar NPs sizes (126–226 nm). As shown in Figure 1 (A-D). In contrast, use of 2 and 3% PVA resulted in slightly larger NPs (160 – 312nm) Figure 2 (A-D).

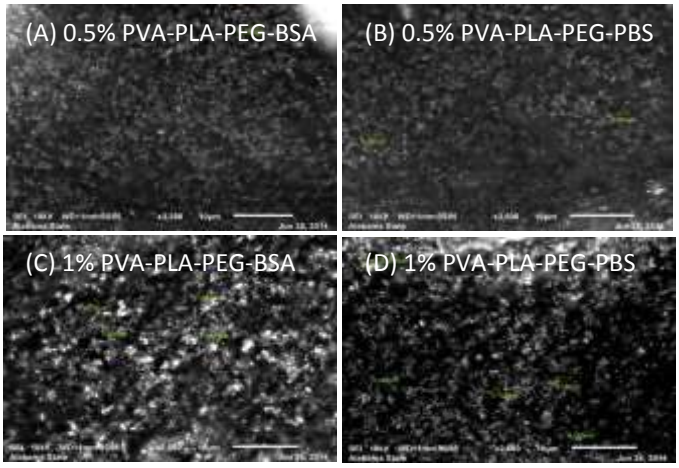

Figure 1. SEM Images of Nanoparticles. (A-B) 0.5% PVA and (C-D) 1.0% PVA formulations.

The data suggests that as the PVA concentration increases NPs formulated with 2 and 3.0% PVA also increased in size. The morphology of NPs irrespective of the PVA concentration formulation were overall spherical with only a small percentage, less than 5%, having serrated or uneven edges.

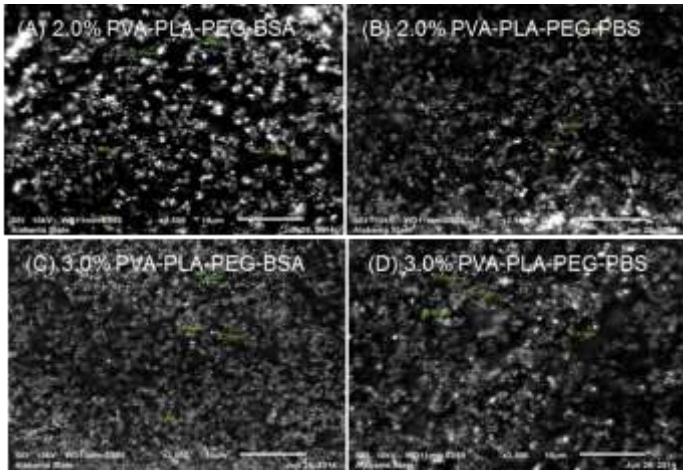

Figure 2. SEM Images of Nanoparticles (A-B) 2.0% PVA and (C-D) 3.0% PVA.

3.3 Effect of PVA concentrations on encapsulation efficiency

We formulated different combination of NPs using 1% PVA to further dissect the effect of changing the polymer and protein ratios, that will be ideal for slow release and high encapsulation of protein. PVA is used as emulsifying agent in NPs fabrication. PVA forms an interconnected network with the polymer at the interface which can affect the degradation profile and size of NPs. In our studies we observed that 0.5, 2 and 3% PVA formulations were less effective for encapsulation of BSA as compared with 1% PVA. As shown in Figure 3, the EE were 42 – 63.4% for 1% PVA. While the 0.5, 2 and 3% PVA resuted in 25.61%, 20.53% and 23.41% EE.

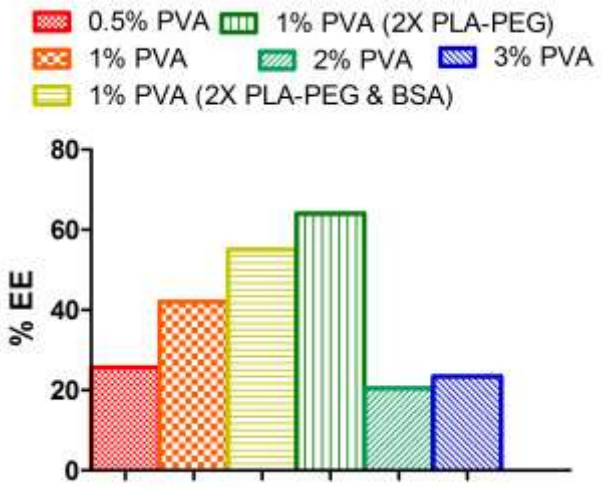

Figure 3. Encapsulation efficiency (EE) of PLA-PEG-BSA using different PVA formulations.

3.4 Cummulative release and UV-Vis of encapsulated BSA

The *in vitro* release profile of encapsulated BSA from PLA-PEG-BSA (Figure 4) shows an intial burst effect followed by a steady and consistent release. The 0.5% PVA formulaion had the highest release of 65.32% BSA; other PVA concentrations had steady releases of BSA with approximately 30% in the early time-points.

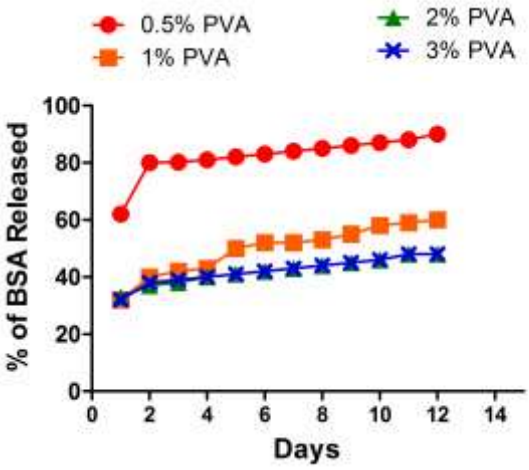

Figure 4. *In vitro* release of BSA from PLA-PEG-BSA formulated with 0.5-3.0% PVA. Release of BSA was performed in PBS buffer (pH 7.4).

In vitro release profiles of nanoparticles formulated with 1% PVA (Figure 5) revealed gradual increment in the cummulative release of protein over the period of 10 days

with the 2x PLA-PEG and protein having the highest release of 47.34% and the 1% normal formulation with 32% release.

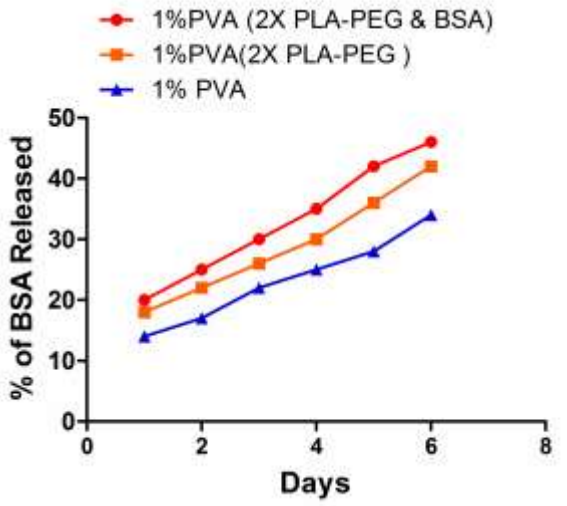

Figure 5. *In vitro* release of BSA from PLA-PEG-BSA NPs formulated with 1.0% PVA.

It is possible that during the course of encapsulation of protein in NPs, adsorbtion of protein to the outer surface of NPs can occur, which could result in the burst effect. To ensure that BSA was successfully encapsulated, we performed UV-vis analysis which revealed the absorbance spectrum of BSA at wavelength of ~285 for proteins. No absorbance was detected for PLA-PEG-BSA and PLA-PEG-PBS NPs (data not shown), thus verifying the successful encapsulation of BSA.

4.0 CONCLUSION

Both SEM and Zetasizer analyses confirmed that PVA helps to create a stable nanoparticle that allows the slow release of protein. Zeta potential revealed that the NPs formulated with 1% PVA were more stable and less aggregated. The PVA coated nanoparticles also had a consistent population ranging in size between 100- 300 nm. The 1% PVA formulations showed that increasing the amount of polymer from 100 mg to 200 mg and protein from 2 to 4 mg had no noticeable affect on the protein release profile. Doubling the polymer resulted in increased encapsulation effieciency by 10%. The 0.5% PVA had the highest burst release in the early hours while other PVA concentrations revealed a small and consistent BSA release. Arguably, the 1.0% PVA seems to be the optimal PVA concentration to use for nanoparticles due to its stability, small consistent BSA release, and nanoparticle size. The different PLA-PEG formulations may be very useful for biomedical applications in terms of vaccine or drug delivery in controling the amount of drug or protein released.

5.0 REFERENCES

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