Synthesis of PLGA nanoparticles with entrapped antibiotic mupirocin

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Abstract: Infections caused by resistant strains of bacteria have reached a critical level calling for increasingly effective delivery system of drugs to prevent and treat these infections. PLGA nanoparticles (NP) with mupirocin encapsulated could be good for better and quicker wound healing process. The aim of the experiment is to develop PLGA NPs with entrapped mupirocin and determine basic characteristic of it. Mupirocin is effective antibiotic in the fight against mentioned infection. For synthesis of these NPs was used emulsion evaporation method. Characterization of prepared NPs consisted of measuring size (141.1 ± 9.3 nm), zeta potential (-21.9 ± 4.6 mV) and morphology by method TEM. Also, we did a release profile of mupirocin from NPs. Loading capacity was calculated as 2.34 µg and entrapped efficiency as 22.4%.

Key Words: PLGA, nanoparticles, synthesis, mupirocin, healing process

INTRODUCTION

Recent studies on improving the healing process of acute and chronic wounds have sought to develop innovative and effective wound dressing materials that could accelerate healing or prevent frequent complications (Rajendran et al. 2018). One such complication is infection with resistant strains of bacteria or sepsis, which could slow or worsen the healing process. The aim of many studies is to deliver the highest concentration of antibiotic directly to the site of action, thereby increasing its efficacy (Vashishth and Kaushik 2017). This can be achieved by nanodelivery systems such as PLGA NPs. The advantages of nanodelivery systems include protection of the drug from degradation and targeted controlled drug release (Shah et al. 2015, Pavitra et al. 2019, Ma and Mumper 2013).

Several types of nanodelivery systems were developed. One of these systems is liposome. Liposomes are frequently used to entrap hydrophilic or hydrophobic antibiotics, have good permeability through cell membranes, low toxicity, and are shown to enhance antimicrobial activity of antibiotic (Schiffelers et al. 2001, Yang et al. 2009). Other nanodelivery system is nanoemulsion. Advantages of this system are biodegradability, biocompatibility, ease of preparation and physical stability can be very useful for delivery of antibiotics (Santos-Magalhaes et al. 2000). Alternatively, polymeric NPs with entrapped or covalently attached drug have been developed to improve stability of the delivery system during storage relative to the other delivery systems (Lockman et al. 2002, Misra and Sahoo 2012). Polymeric NPs can protect the drug against degradation in the body and they have improved body or tissue tolerance (Müller et al. 2000, Kalhapure et al. 2015).

Mupirocin (MUP) was isolated in 1971 from *Pseudomonas fluorescens* (Fuller et al., 1971) and was proven to be effective in treating skin infections, especially impetigo, burns, folliculitis and foot ulcers (Perumal et al. 2014). MUP is a hydrophobic topical antibiotic used in the form of topical ointments to treat these conditions. This antibiotic has documented antimicrobial activity against

resistant strain bacteria *Staphyloccocus aureus* (Mahalakshmi and Sankar, 2020). MUP inhibits bacterial protein synthesis by specific and reverse binding to isoleucine tRNA synthetase. This causes isoleucine not to be incorporated into the emerging proteins (Okur et al. 2019, Rode et al. 1989). After intravenous or oral administration, mupirocin is metabolized to an inactive form (monic acid) and excreted into the kidneys (Alcantara et al. 2019).

The goal of the study was to synthesis PLGA NPs with entrapped mupirocin. PLGA polymer was chosen because it is good polymer for encapsulating hydrophobic drugs and is widely used in the pharmaceutical industry. Basic characteristics such as morphology, size, size distribution, surface charge, and release kinetics were determined. Ultimately, these NPs will be incorporated in dressings and their efficacy in wound healing will be assessed *in vivo*.

MATERIAL AND METHODS

Chemicals and materials

Poly(lactic-co-glycolic acid), mupirocin (MUP), polyvinyl alcohol (PVA), trehalose dihydrate obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethyl acetate and acetonitrile were obtained from EMD Chemicals Inc. (Gibbstown, NJ, USA). Nanopure water obtained using Nanopure Diamond and 0.2 µm Barnsted D3750 Hollow Fiber Filter (Barnsted International, Dubuque, IA, USA).

Synthesis of PLGA NPs with entrapped mupirocin

Synthesis of NPs was achieved by emulsion evaporation method. Aqueous solution of 2% PVA was prepared while stirring (400 RPM) at 60 °C. Then 7 ml ethyl acetate was added to the aqueous phase. An organic phase was formed by dissolving 220 mg of PLGA and 11 mg MUP in 5 ml of ethyl acetate under stirring (220 RPM). The organic phase was added to the aqueous phase drop wise and the formed emulsion was microfluidized (Microfluidizer M110P, Microfluidics, MA) 4 times at 30,000 psi in an ice bath. Next, ethyl acetate was evaporated on Rotovap (R-124 rotary evaporator Buchi Inc., New Castle, DE) for 60 min under vacuum (40 mmHg) and stirring at 60 RPM. NPs were purified by dialysis with a 300 kDa Spectra/ POR CE membrane (Spectrum Rancho, CA, USA) in water to remove free PVA. After two days of dialysis, 650 mg trechalose (1:1 w/w) was added to the NPs suspension prior to freeze-drying. The sample was freeze-dryed with Labconco (2.5 plus freezone, Labconco Corporation, MO, USA) for two days. Finally, the sample was stored at -20 °C for further analysis. Empty NPs were prepared by the same protocol without added mupirocin in the organic phase.

Characterization of PLGA-MUP NPs

Morphology

Morphology of loaded and empty NPs was determined by Transmission Electron Microscopy (TEM) assessed using a JEOL JEM-1400 transmission electron microscope (JEOL USA Inc., Peabody, MA, USA). The lyophilized sample was re-suspended in water at a concentration of 5 mg/ml and sonicated in a water bath for 5 minutes. One droplet (3 µl) of sample was placed on the glow discharged 300 mesh carbon film grid (TEM-CF300-cu) obtained from Sigma-Aldrich (St. Louis, MO, USA). After 2 minutes, a filter paper was used to remove excess liquid and 2% UA (uranyl acetate) was added as a contrast agent.

Size and zeta potential

Lyophilized samples were dissolved in water at a concentration of 0.25 mg/ml, placed into disposable capillary cell (Malvern Panalytical Inc., Westborough, MA, USA) and the size and zeta potential was measured by DLS using the Malvern Zetasizer Nano ZS (Malvern Instruments Inc., Southborough, MA, USA).

Release of mupirocin from NPs

Lyophilized NPs were dissolved in water at a concentration of 5 mg/ml. Then, 15 ml of the suspension was placed into a dialysis tube (Spectra/por Dialysis membrane, Mw 300 kD) and suspended in water. The sample was stored at 37 °C and at 95 rpm in an incubator shaker C25 KC (New Brunswick Scientific Inc., Edison, NJ, USA). At each time interval (0, 2, 4, 6, 24, 48, 72 hours) 0.2 ml sample was taken out of the dialysis tube, mixed with acetonitrile (ACN) at 8:2 ACN: water v/v

and was placed in vortex for 30 minutes shaking. After that, the sample was centrifuged with Allegra 64R centrifuge (Beckman Coulter) at 20 000 rpm for 20 min at 10 °C. Supernatant was removed and the MUP measured by UV spectrometry at 210 nm by UV-Vis spectrophotometer (Genesys 6, ThermoFisher Scientific, Waltham, MA). The MUP concentration was determined based on a standard curve made under the same conditions.

RESULTS AND DISCUSSION

The empty and loaded NPs were smaller than 200 nm and were negatively charged, with loaded NPs more negatively charged (-21.9 ± 4.6 mV) than empty NPs (-4.95 ± 13.5 mV). Both types of particles were polydispersed as indicated by the high PDI (Table 1) and the TEM images (Figure 1).

Table 1 Size and zeta potential

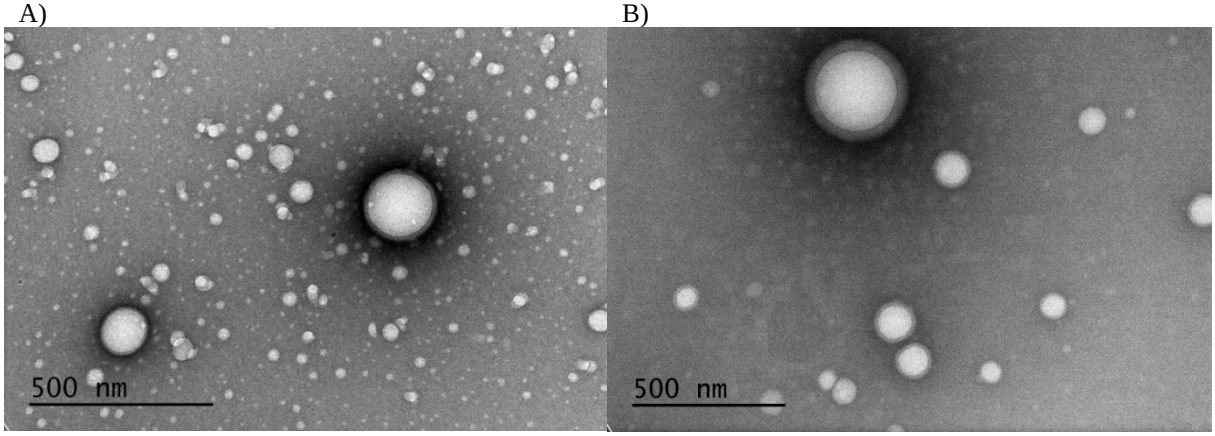
Sample	Size (nm)	Zeta potential (mV)	PDI
Control (empty NPs)	190.7 ± 19	-4.95 ± 3.5	0.218 ± 0.018
Loaded NPs	141.1 ± 9.3	-21.9 ± 4.6	0.336 ± 0.013

Morphology

Entrapped NPs had a spherical shape. There are no differences between the morphology of entrapped NPs (B) and control (empty) NPs (A) and both types of particles were polydisperse.

Figure 1 TEM pictures of PLGA NP*s with PVA (A) empty NPs, (B) with entrapped Mupirocin*

A) B)



Loading capacity (LC)

The loading capacity was calculated using the equation:

$$LC = \frac{\text{amount of MUP in NPs (µg)}}{\text{amount of powder NPs (mg)}}$$

Loading of NPs with entrapped mupirocin purified by dialysis was 2.34 µg MUP/mg powder.

Entrapped efficiency (EE)

Entrapped efficiency was calculated using the equation:

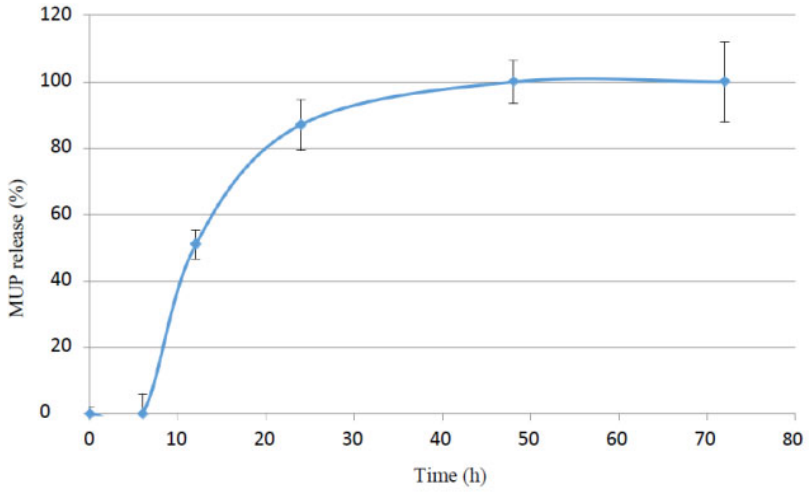
$$EE(\%) = \frac{\text{amount of MUP in the system on start (µg)} - \text{free MUP}}{\text{amount of MUP in the system on start (µg)}} \times 100$$

Entrapment efficiency was found to be 22.4% for entrapped NPs purified by dialysis.

Release study

Release profile was measured over a period of 72 hours by UV spectrometry. Most of MUP (x%) was released in 24 hours and 100% was released in 48 hours (Figure 2).

Figure 2 Release study of mupirocin over time



CONCLUSION

PLGA NPs with entrapped MUP were synthesized by emulsion evaporation method in the presence of PVA as a surfactant. According to the results of the study, these NPs are small, negatively charged and can be efficiently loaded with MUP. The controlled release of this antibiotic over two days could prove beneficial in wound healing. Next step of this research consists of testing the NP cytotoxicity *in-vitro* and their antibiotic efficacy *in vivo* as measured by wound healing in postoperative conditions.

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