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RESEARCH PAPER

Synthesis and characterizations of gentamicin-loaded poly-lactic-co-glycolic (PLGA) nanoparticles

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Abstract Poly-lactic-co-glycolic acid (PLGA) was mixed with gentamicin sulfate via a double emulsion method, resulting in gentamicin-loaded PLGA nanoparticles that exhibited excellent antibacterial properties and great potential in fabricating smart wound dressings integrated with a drug delivery system. The nanoparticle morphologies, particle degradation rates, drug release profiles, and antibacterial properties were investigated using scanning electron microscopy (SEM), dynamic light scattering (DLS), ultraviolet–visible spectroscopy (UV–vis), and disk diffusion method. Nanoparticles prepared at different PLGA concentrations exhibited different release profiles that were determined by multiple release mechanisms including diffusion, osmotic pumping, and nanoparticle degradation. The antibacterial activities were measured using a disk diffusion method indicating that various nanoparticles loaded with antibiotics can control bacterial infection to some degree proving that nanoparticles used in this paper can be used in the pharmaceutical industry. The results suggested that drug release properties of gentamicin loaded PLGA nanoparticles can be affected by PLGA concentrations and PVA concentrations in the particle synthesis, providing a guidance in preparing gentamicin-loaded PLGA nanoparticles for topical antibiotics delivery applications. The nanoparticles with a spherical and uniformly porous structure were prepared with a PLGA concentration of 0.0167 g/ml and a PVA concentration of 12%, resulting in the highest average gentamicin release rate and excellent antibacterial activities. Four release mechanisms (diffusion through polymer, diffusion through pores, osmotic pumping, and nanoparticle degradation) primarily determined the gentamicin release process.

Keywords Poly lactic-co-glycolic acid; Nanoparticles; Polyvinyl alcohol; Particle morphology · Particle size · Particle degradation · Drug delivery

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Introduction

Wound treatment against bacterial infection has always been a concern for medical professionals. The use of antibiotics has been proven to be the most effective and fastest approach for controlling

bacterial infections. Therefore, finding an efficient and timely way to deliver antibiotics to the wounds is critical for successful wound care and management (Stebbins et al. 2014). There are usually three approaches to deliver antibiotics: taken by mouth (orally), injection into veins (intravenously), and application to skin (topically). Because the topical antibiotics only act on wound sites, some typical unwanted side effects, such as nausea or diarrhea, can be avoided. In addition, it is evident that the use of topical antibiotics can reduce the possibility of bacterial resistance (Wei 2012). Recently, topical antibiotics have been encapsulated in a nanoparticle, and hence, the bioactivity of antibiotics can be preserved for a given time, resulting in scalable production. Nanoparticles can deliver drugs through blood capillaries and allow for access into cells (Cho et al. 2008), resulting in a more effective delivery than other applications of topical drugs. In addition, the drug release rates in nanoparticles can be efficiently tuned by controlling particle size distributions, and particle morphology that are primarily determined by the materials and synthesis methods of nanoparticles.

Different materials have been reported in the synthesis of nanoparticles used in medicines, including chitosan, gelatin, polycaprolactone, and poly-lactic-co-glycolic acid (PLGA) (Wei 2012). PLGA has been attractively used in the pharmaceutical industry due to its superior biodegradability, biocompatibility, and nontoxicity (Makadia and Siegel 2011). When PLGA is introduced to the circulatory system of human body, it can be decomposed into carbon dioxide and water, suggesting no harm to the body (Panyam and Labhasetwar 2003). PLGA nanoparticles have been used to deliver drugs in cells and tissue engineering. PLGA nanoparticles can be loaded with protein, peptides, and low molecular weight compounds for many therapeutic applications (Panyam and Labhasetwar 2003). Jiang et al. used PLGA nanoparticles as an antibiotic delivery system to control bacterial infection, demonstrating good antibacterial activities fighting against common bacterial infections (Jiang et al. 2018). The efficiency of drug release to targeted positions can be dramatically increased by using therapeutic PLGA nanoparticles. In addition, PLGA nanoparticles can serve as a sustainable system to deliver genes or antibiotics with a stable release rate (Cho et al. 2008).

There are two methods commonly used to synthesize PLGA nanoparticles. The first method is a chemical process including mini-emulsion polymerization (Landfester et al. 2003), emulsion solvent evaporation (Lee et al. 2005), compressed antisolvent along with emulsion solvent evaporation method (Imbuluzqueta et al. 2011), and interfacial polymerization (Gao et al. 2004). The second method is a physicochemical process including multiple emulsion techniques (Fangueiro et al. 2012), emulsion solvent diffusion (Cohen-Sela et al. 2008), layer by layer process (Hua et al. 2002), and spray drying (Iqbal et al. 2015). For example, mini-emulsion polymerization or interfacial polymerization is a step-growth polymerization process. In this method, the polymerization occurs at the interface between two immiscible phases, which makes difficult to control PLGA nanoparticle size. Another example is the water in oil emulsion solvent diffusion method that may produce the nanoparticles good for hydrophilic drugs, but shows some drawbacks, such as high polydispersity and irregular morphology. Among these methods, the emulsion evaporation method is promising in producing nanoparticles that are spherical, regular, and small in diameter, suggesting a feasible method for synthesizing PLGA nanoparticles. The emulsion solvent evaporation technique was developed by Ogawa in 1998 (Ogawa et al. 1988), and then a variety of methods based on the Ogawa technique have been developed. One example is a double emulsion evaporation method that has been demonstrated effectively in synthesizing PLGA nanoparticles for hydrophilic-drug entrapment. The double emulsion evaporation method is an easy-to-control process and cost-effective, requiring no special instruments (Ruan et al. 2002). Another similar example is a water-in-oil-in-water approach, a small amount of water (*w1*) is dispersed in an oil or organic phase (*o*) leading to a primary emulsion (*w1/o*). Then, the primary emulsion is dispersed in another continuous aqueous phase (*w2*) forming large droplets, resulting in a double emulsion to develop PLGA nanoparticles.

Previous experiments have been conducted to investigate the impact of pH, sonication time, temperature, antibiotics concentrations, and stirring rates on the properties of PLGA nanoparticles. Posadowska et al. studied the impact of antibiotic concentrations on the properties of PLGA nanoparticles such as particle size, shape, and drug solubilization (Posadowska

et al. 2015). Abdelghany et al. reported that when the pH of aqueous phase in the synthesis was increased from 5.6 to 7.4, the antibiotics became less hydrophilic, and hence, the antibiotic molecules were more likely entrapped in the PLGA nanoparticles (Abdelghany et al. 2012). In addition, Flores et al. found that the increase of stirring rates in the particle synthesis decreased the size of the antibiotic encapsulated PLGA nanoparticles as well as the antibiotic release rates (Flores et al. 2016). Temperature effects illustrated by Kwon et al. showed that nanoparticle size would decrease as the temperature increasing (Kwon et al. 2001).

On the other hand, antibiotic release by nanoparticles is critical in developing nanoparticle drug delivery system. Virto et al. used a dialysis method to study drug release mechanisms and suggested a kinetics model to understand the release mechanisms (Virto et al. 2007). The main release mechanism in the kinetics model was the breaking of ester bonds called heterogeneous hydrolytic degradation (Virto et al. 2007). Budhian et al. studied PLGA nanoparticles loaded with haloperidol and suggested a release mechanism using a govern equation that was derived from semi-empirical equations based on diffusion (Budhian et al. 2008). A recent study reported an encapsulation-free mechanism for controlled release, providing the underlying mechanism due to short-range electrostatic interactions between the proteins and the nanoparticles (Pakulska et al. 2016). In addition, previous work has shown that multiple release mechanisms occur simultaneously, and the primary mechanism could be switched during the release (Fredenberg et al. 2011). Different nanoparticles may have various release profiles and multiply release mechanisms. As a result, defining a general release mechanism in PLGA nanoparticle release process can be challenging.

Although PLGA has been widely studied in drug delivery systems, to our best knowledge, the effects of PLGA and surfactant concentrations on nanoparticle properties have not been systematically investigated previously. It is especially unknown yet that how the PLGA and surfactant concentrations on the performance of drug release from PLGA nanoparticles. In addition, although the antibiotic release mechanisms using the PLGA nanoparticles have been previously discussed, few works paid attention to the relationship of antibiotic release mechanisms and nanoparticle degradation processes. This paper demonstrated a double emulsion method of fabricating antibiotic-encapsulated PLGA nanoparticles that showed uniform porous nanostructures, time-dependent drug release and particle degradation profiles, and good antimicrobial performance. Gentamicin sulfate was chosen as the antibiotic encapsulated in the PLGA nanoparticles because gentamicin is a broad-spectrum antibiotic for both gram-positive and gram-negative bacteria. Synthesis parameters including PLGA concentrations and polyvinyl alcohol (PVA) surfactant concentrations demonstrated significant impacts on the particle size distribution and morphology. It was found that the PLGA and PVA concentrations had significant effects on release profiles, morphology, and size distribution of the nanoparticles. The degradation rate of PLGA nanoparticles and the release rate of gentamicin were determined via using a UV–vis spectroscopy. The nanoparticles synthesized at 9% and 12% PVA concentrations have spherical shapes and uniform porous structures on particle surfaces compared to those nanoparticles synthesized with PVA below 7%. The nanoparticles synthesized at different PLGA concentrations have various release profiles due to multiple combinations of release mechanisms such as diffusion, osmotic pumping, and particle degradation. The gentamicin-loaded PLGA nanoparticles demonstrate antibacterial activities when *Escherichia coli* was used in a disk diffusion method. The PLGA nanoparticles loaded with gentamicin were tested in an *E. coli* inhabitation study, showing excellent antibacterial activities.

Experimental section

Materials

Hydrolyzed polyvinyl alcohol (99 + %, Mw = 89,000–98,000, $[\text{-CH}_2\text{CHOH-}]_n$, USA), gentamicin sulfate salt powder (99 + %, G1264-5G), the poly-lactide-co-glycolic acid (lactide, glycolic 75:25, Mw 4000–15,000, $[\text{C}_3\text{H}_4\text{O}_2]_x[\text{C}_2\text{H}_2\text{O}_2]_y$, RG502H), and anhydrous dichloromethane (Mw = 84.93, 99.8%, CH_2Cl_2) were purchased from Sigma-Aldrich. HPLC-grade submicron-filtered water (pH = 7, 7732–18-5, W5-4, USA) was purchased from Fisher Chemical.

The chemicals were used without further purification. *Escherichia coli (E. coli)* ATCC25922 (1×10^4 cfu/pellet) was purchased from ATCC (Manassas, USA). Luria broth (LB) media, BBL Muller Hinton II Agar, used to grow *E. coli* was purchased from Becton, Dickinson and Company, Sparks, MD.

Gentamicin-loaded PLGA nanoparticle synthesis

The PLGA nanoparticles containing gentamicin were synthesized via a double emulsion evaporation method adopted from Astete in 2006 (Imbuluzqueta et al. 2011). Three solutions of different phases used in the emulsion method were firstly prepared, including an oil phase (*O*), a water phase 1 (*W1*), and a water phase 2 (*W2*). PLGA powder was first dissolved in dichloromethane. A range of masses of PLGA from 80, 90, 100, 110, to 120 mg were dissolved in 6 ml dichloromethane (DCM), resulting in PLGA concentrations of 0.0133 g/ml, 0.015 g/ml, 0.0167 g/ml, 0.0183 g/ml, and 0.02 g/ml. The PLGA solution was the oil phase (O).

Polyvinyl alcohol (PVA) was a surfactant and dissolved in distilled water. The concentrations of PVA solutions used in the experiment were 3%, 5%, 7%, 9%, and 12% (weight/volume). The solution was stirred at 40 °C for overnight. Twenty milligram gentamicin sulfate was dissolved in 200 µl distilled water. A Whatman quantitative 90 mm filter paper (VWR®) was used to filter out any undissolved powder. The filtered solution was sonicated via a sonic dismembrator (Fisherbrand™-Model 505) at 35% amplitude for 30 s to improve the consistency of the PVA solution. Seventy-five microliters of PVA solution was mixed with the gentamicin solution. The mixing solution was kept in a refrigerator overnight to completely dissolve, resulting in the water phase 1 (*W1*). PVA solution was used as the water/aqueous phase 2 (*W2*).

After the three solutions of different phases ([*O*], [*W1*], and [*W2*]) were prepared, they were utilized in a double emulsion evaporation method consisting of four steps, resulting in gentamicin encapsulated PLGA nanoparticles. The four steps are described in Fig. 1 as below.

Step 1: First, (*O*) was mixed with (*W1*) for making a primary emulsion solution.
Step 2: The (*O*) was mixed with (*W1*) and the mixture was sonicated for 3 min at 35% amplitude yielding a primary emulsion solution. This was to disperse the mixture to have small nanodroplets.
Step 3: The primary emulsion was mixed with (*W2*), leading to a double emulsion of nanodroplets.
Step 4: After the double emulsion solution was stirred for 4 h, nanoparticles precipitated after the solvent (dichloromethane) had diffused from (*O*) to (*W2*) resulting in the PLGA nanoparticles precipitating around (*W1*) (Bilati et al. 2005). The surfactant remained at the interface during the diffusion process and helped nanoparticles encapsulate antibiotics. The PLGA nanoparticles were formed after the solvent from ***o*** completely diffused to (*W2*).

The mixture was divided in ten 15-ml plastic tubes, resulting in 5 ml of solution in each tube for further particle characterizations. Tubes were put into centrifugation at 6000 rpm for 10 min. Each tube was decanted and rinsed with 5 ml dissolute water to wash away any residue of nonparticulate PLGA or PVA. The rinse was performed three times in each tube, and then each tube was allowed to dry for 10 h at room temperature.

Material characterization

The morphology and size distribution of PLGA nanoparticles made at different PLGA and PVA concentrations were investigated via scanning electron microscopy (SEM) (JEOL JSM-6500F field Emission Scanning Electron Microscopy) and dynamic light scanning (DLS) (Malvern Zetasizer Nano ZS). In sample preparation for SEM imaging, the nanoparticles were first dispersed in dissolute water, and then dropped casted onto a silica wafer that was left to set until the water had completely evaporated. The silica wafer was coated with 3 nm of gold, and then examined via SEM to study the particle morphology. In a DLS measurement, 3 ml of highly dilute nanoparticle solution was put into a cuvette for evaluation of particle size distribution. Five samples of particle solutions were measured in the DLS. A size distribution profile was compiled from the five DLS measurements for each particle solutions. The SEM and DLS measurements were used to determine the highest yield of PLGA nanoparticles by PVA concentrations. An optimal PVA concentration was determined at

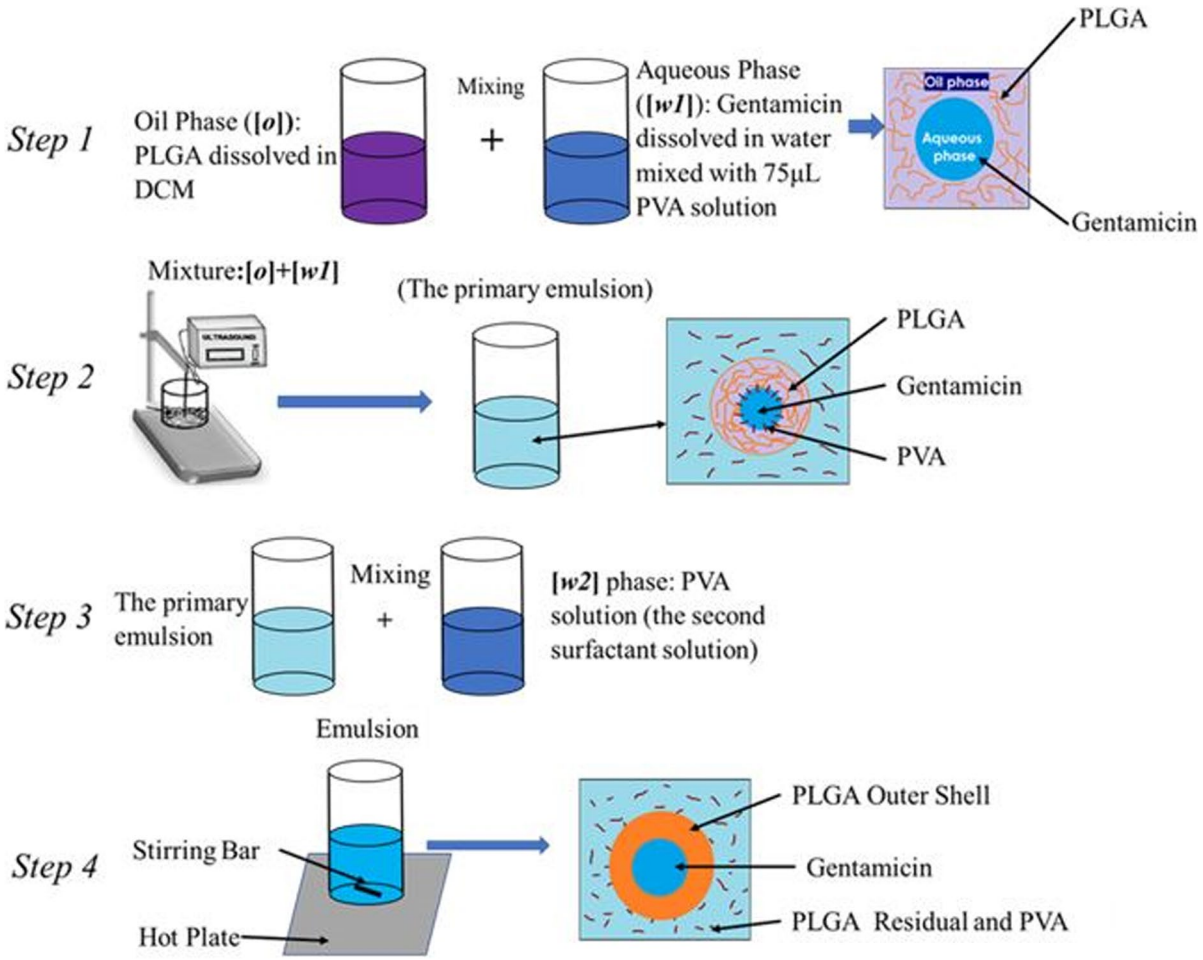

Fig. 1 Schematic illustration of the gentamicin loaded PLGA nanoparticle synthesis

high yield and uniform morphology of the particles that were further used to study nanoparticle degradation and gentamicin release rates which were both functions of PLGA concentrations.

Gentamicin release rate

The gentamicin was expected to release over time. A method adopted by Xiong et al. (Xiong et al. 2014) was used to study the release rate of the nanoparticles obtained at the optimal PVA concentration and different PLGA concentrations. An UV–vis spectroscopy (Agilent Cary 4000) was used to measure gentamicin concentrations in solution over time. Before carrying out the release rate determination experiments, a calibration curve of gentamicin was obtained using a range of gentamicin solutions with known concentrations. The samples used in the UV–vis absorbance measurements were the liquid solutions after centrifuging because the gentamicin was dissolved in the water after it was released from the nanoparticles. The liquid samples were measured with UV–vis, and the absorbance at 196 nm was used to quantify the gentamicin concentrations released from the nanoparticles according to the calibration curve. The release rate of gentamicin was presented as a function of time from $t = 0$ h to $t = 10$ h. Five samples for each nanoparticle solutions (at different concentrations) were tested and the release rate data were reported based on the five sample measurements.

PLGA nanoparticle degradation

A method adopted by Soppimath (Soppimath et al. 2001) to assess particle degradation was used to determine the mass change of PLGA nanoparticles in solution as a function of time. The selected PLGA nanoparticles at the optimal PVA concentration were chosen in the measurements. The obtained particle suspension was further diluted in dissolute water and kept at room temperature to allow particle degradation. Suspension samples were taken every 2 h, from $t=0$ h to $t=10$ h, to measure the mass change at particle degradation. The sample solutions were centrifuged, decanted, and dried for 10 h. The mass of the precipitate was measured. The ratio of the remaining weight to the original weight was calculated in Eq. 1 (Astete and Sabilov 2006):

$$W_i = \frac{w_i}{w_0} \tag{1}$$

where the w_0 is the weight of precipitate at 0 h; w_i is the weight of collected precipitate after the solution was kept in the tube for a period of time; and W_i is the weight ratio of remaining precipitate compared to the original precipitate.

The degradation experiments were repeated for nanoparticles obtained at different PLGA concentrations (0.0133 g/ml, 0.015 g/ml, 0.0167 g/ml, 0.0183 g/ml, 0.02 g/ml). The weight ratio was calculated for each concentration of nanoparticle solutions. Five samples for each nanoparticle (at different concentration) solutions were tested and the release rate data were reported based on the five sample measurements.

Antibacterial testing

An agar diffusion method was used in testing antibacterial properties of the gentamicin-loaded PLGA nanoparticles obtained at the optimal PVA concentration and different PLGA concentrations (0.0133 g/ml, 0.0150 g/ml, 0.0167 g/ml, 0.0183 g/ml, and 0.0200 g/ml) (Posadowska et al. 2014). A premade *E. coli* ATCC25922 pellet (1×10^4 cfu/pellet) was mixed in 200 ml LB medium. The solution was incubated at 37° C for 24 h. An inoculation loop was used to transfer the bacteria to an LB streak plate. Then, the bacteria culture was placed in an incubator to grow for 24 h at 37° C. BBL Muller Hinton (MH) II Agar was used to prepare MH agar plates. Thirty-eight grams agar powders were dissolved in 1 l of DI water. The mixture was then heated on a hot plate with a stirring rate at 450 rad/min to reach complete dissolution. After the MH agar solution was autoclaved at 121 °C for 15 min, it was distributed into a number of petri dishes with 25 ml solution in each dish, and the petri dishes were kept at room temperature overnight to dry. The dry MH agar plates were collected for the antibacterial testing. Five milliliters DI water was mixed with about 45 mg gentamicin-loaded PLGA nanoparticles. The mixture was sonicated at 35% amplitude for 1 min, resulting a uniform dispersion of the PLGA nanoparticles. A dispersion of PLGA nanoparticles without gentamicin was used as a control sample in the testing. A colony of *E. coli* was taken from the LB agar plate and streaked in a 6 MH agar plate that was used to test the PLGA nanoparticles. There were totally 6 MH agar plates for the five PLGA concentration (0.0133 g/ml, 0.0150 g/ml, 0.0167 g/ml, 0.0183 g/ml, and 0.0200 g/ml) as well as the control nanoparticles, respectively. In each MH agar plate, 5 wells in a diameter of 3 mm were cut and 50 µl of a nanoparticle solution was placed in each well. The agar plates were kept in an incubator at 37° C for 24 h. The diameter of each bacterial-free (inhibition) zone (each well) in the agar plates were measured after 24 h and the average from the five wells were reported for antibacterial activity. The data are reported with five sample measurements for each nanoparticle. The error bars were calculated with the five measurements for each nanoparticle.

Results and discussion

PLGA nanoparticle size and morphology

PVA concentration

PVA was a surfactant in the double emulsion method and had demonstrated a significant impact on the size and morphology of nanoparticles when gentamicin was encapsulated in the nanoparticles.

The morphology and size of the nanoparticles obtained at different PVA concentrations were assessed via SEM images and DLS measurements.

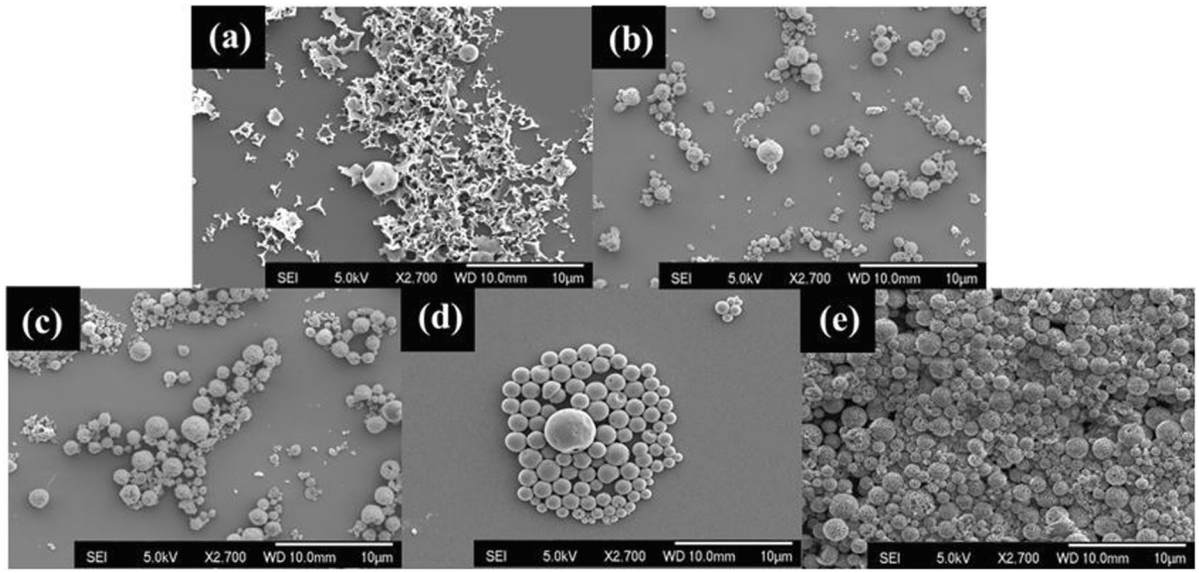

Fig. 2 Representative SEM images of the PLGA nanoparticles made at different PVA concentrations (v/w): **a** 3%; **b** 5%; **c** 7%; **d** 9%; **e** 12%

Figure 2a–e shows SEM images of the nanoparticles with different morphologies, especially different uniformity. Particle morphology plays an important role in drug lease of nanoparticles. Future work includes development of computational models that help explore the effect of nanoparticle morphology on drug release mechanism at different release stages.

The SEM images suggested that the nanoparticles synthesized with 9% and 12% PVA concentrations showed roughly uniform spherical structures while there were almost no spherical particles obtained at 5% or below PVA concentrations. The results demonstrated a key role of the PVA (surfactant) in the nanoparticle formation, which was in a good agreement with previous research (Champion et al. 2007). PVA has two different ligands; one is hydrophobic, and the other one is hydrophilic. When the ***w1-o-w2*** emulsion solution was formed, the PVA was aligned in a fashion where the hydrophobic parts bonded with the oil phase (PLGA), whereas the hydrophilic parts attached to the water phase. The PVA was able to reduce the surface tension between the two liquid phases, and hence prevented phase separation (Ficheux et al. 1998). The water-PVA-oil mixture (as shown in step 4 of Fig. 1) eventually developed into nanoparticles after the organic solvent for dissolving PLGA had diffused to the outer solution matrix. Therefore, the increase in the PVA concentration helped enhance the nanoparticle formation, resulting in more uniformly spherical particles. Figure 3a–e shows the DLS size distribution of nanoparticles made at different PVA concentrations. The surface of nanoparticles was decorated with pore structures. The formation of these pores was attributed to the diffusion of PVA during the evaporation process leaving porous channels on the surface of PLGA nanoparticles (Zhu et al. 2014).

The DLS size distributions showing the average particle sizes of PLGA nanoparticles made at 3%, 5%, 7%, 9%, and 12% PVA concentrations were 32 ± 2.5 nm, 350 ± 30.4 nm, 980 ± 140.9 nm, 1050 ± 117.4 nm, and 1000 ± 140.9 nm, respectively. The results suggested that large particles were obtained prominently at high PVA concentration. On the other than, the SEM images revealed that the particle synthesis was not sufficient at 3% or 5% PVA because very few spherical particles were identifiable. Therefore, the small particle sizes given by the DLS measurements of 3% and 5% PVA were probably associated with suspended and unreacted polymers in the solutions. Figure 3f shows a relationship between the PVA concentration and the average particles size, indicating that the PVA played an important role in the particle formation. The nanoparticle size was increased with an increase in the PVA concentration

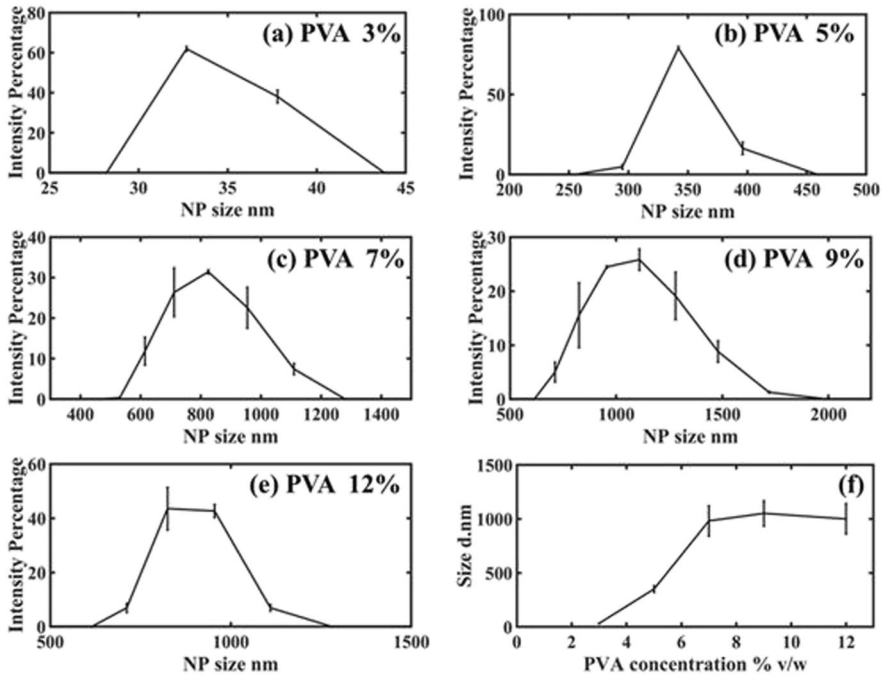

Fig. 3 **a-e** show DLS size distributions of nanoparticles at different PVA concentrations (v/w): **a** 3%; **b** 5%; **c** 7%; **d** 9%; **e** 12%. The data are reported with five sample measurements for each nanoparticle. The uncertainties were calculated with the five measurements for each nanoparticle. **f** The relationship between the PVA concentration and the nanoparticle average size. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle.

from 3 to 7%, followed by no significant changes when the PVA concentration was higher than 9%, suggesting that the PVA concentration above 9% was not a dominant factor any more in the particle formation.

The results of particle size and morphology suggested that the PVA concentration should be higher than 9% to obtain uniform and spherical PLGA nanoparticles. In a comparison of particle morphologies shown in Fig. 2, the spherical particles showed excellent uniformity when the PVA concentration was 12%. Therefore, a 12% was decided as an optimal PVA concentration in the preparation of PLGA nanoparticles for further investigation on particle degradation and drug release profiles in this study.

PLGA concentration

When the PVA concentration was fixed at 12%, a range of PLGA nanoparticles were prepared at different PLGA concentrations. Particle morphology was examined using SEM and the images are shown in Fig. 4a–e.

Porous structures were found in all PLGA nanoparticles, which would be beneficial for drug release. The porous structure was primarily due to the hydrophilicity of PVA that diffused from the nanoparticles to liquid mixtures during PVA evaporation process, resulting in porous structures on the surface of PLGA nanoparticles (Zhu et al. 2014). The pore size was decreased with an increase of PLGA concentration. Figure 5a–e shows the size distributions obtained in DLS measurements of nanoparticles made at different PLGA concentrations.

The average sizes of nanoparticles synthesized at 0.0133 g/ml, 0.0150 g/ml, 0.0167 g/ml, 0.0183 g/ml, and 0.0200 g/ml PLGA were 1500 ± 102.3 nm, 1600 ± 123.1 nm, 1000 ± 140.9 nm, 1800 ± 160.7 nm, and 2400 ± 217.8 nm, respectively. Figure 5f shows the particle size as a function of PLGA concentration and indicated that the particle size generally increased with an increase in PLGA concentration, but there was a short span in which size decreased with increasing concentration (0.015 to 0.0167 g/ml).

The smallest nanoparticles were obtained at 0.0167 g/ml PLGA used in the synthesis. Interestingly, the smallest nanoparticles made at 0.0167 g/ml PLGA showed smallest pores.

Nanoparticle yield was defined as the overall weight of nanoparticles that were synthesized in every batch divided by the mass of PLGA precursor used in the synthesis. Figure 6 shows the nanoparticle yield percentages as a function of PLGA concentrations.

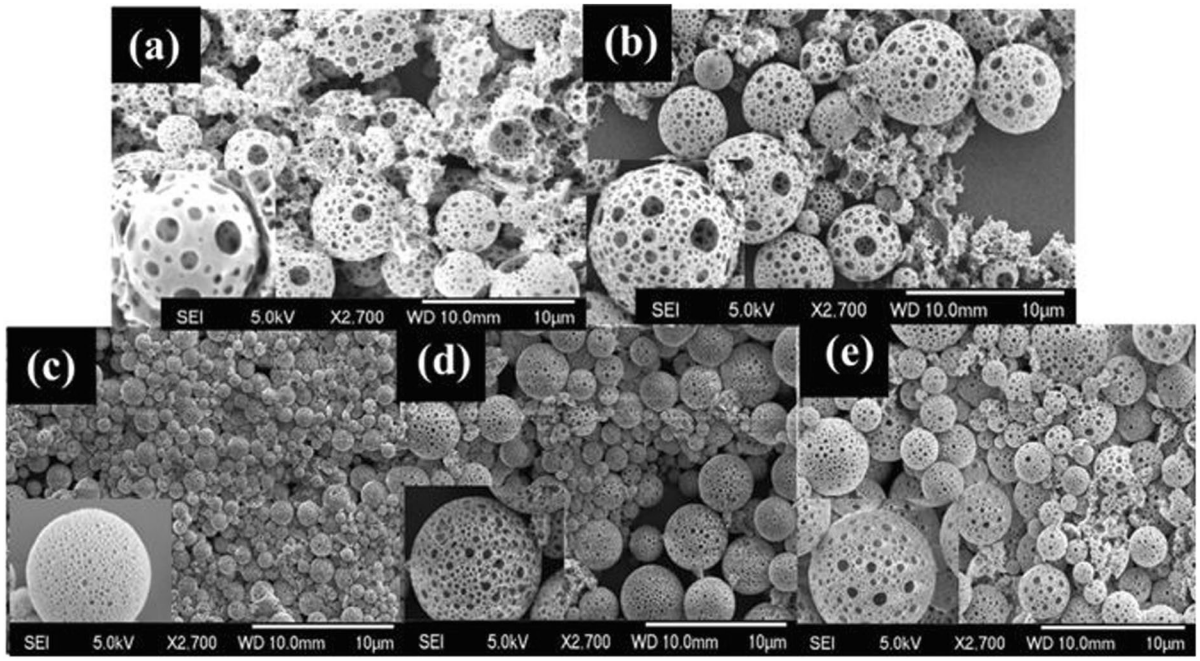

Fig. 4 SEM images of PLGA nanoparticles with different PLGA concentrations: **a** 0.0133 g/ml; **b** 0.0150 g/ml; **c** 0.0167 g/ml; **d** 0.0183 g/ml; **e** 0.0200 g/ml

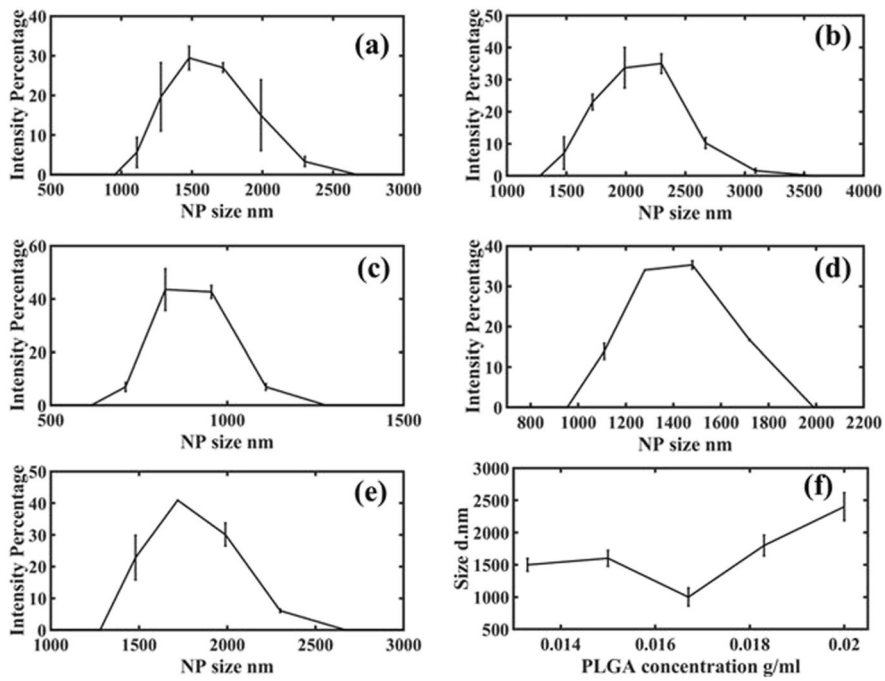

Fig. 5 **a-e** shows DLS size distributions of nanoparticles with different PLGA concentrations: **a** 0.0133 g/ml; **b** 0.0150 g/ml; **c** 0.0167 g/ml; **d** 0.0183 g/ml; **e** 0.0200 g/ml. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle. **f** shows relationship between PLGA concentrations of and particle size. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle.

The nanoparticles yields were 68.34%, 58.89%, 96.33%, 32.12%, and 38.16% when the PLGA concentration increased from 0.0133 to 0.02 g/ml, suggesting the highest yield obtained at 0.0167 g/ml PLGA. In a summary, 0.0167 g/ml PLGA concentration and 12% PVA produced uniform and spherical

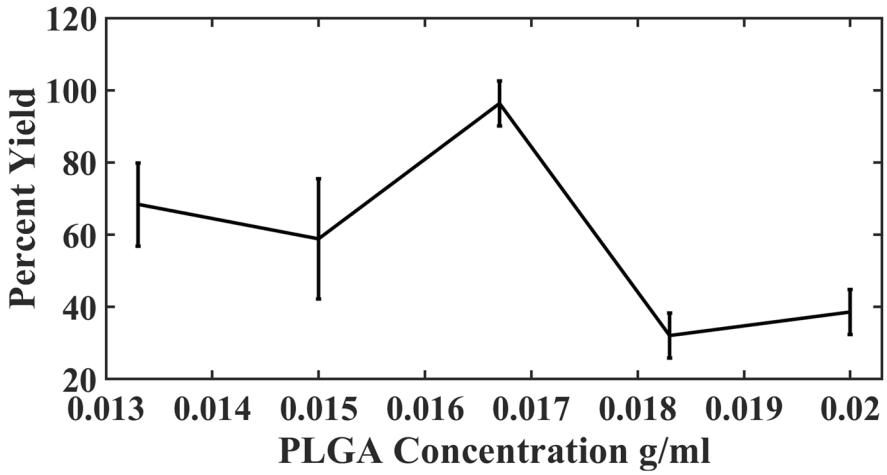

Fig. 6 Relationship between nanoparticles yield percentage and PLGA concentration. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle

nanoparticles that show high yield and small pores on surfaces.

Gentamicin release of PLGA nanoparticles

The PLGA nanoparticles were expected to release gentamicin over time. The release rate was assessed by measuring the change of gentamicin concentrations in nanoparticle solutions from $t=0$ h to $t=10$ h.

The concentration of released gentamicin was calculated using a reference of a linear calibration curve developed with a range of known gentamicin solutions. The gentamicin release profiles for the nanoparticles obtained at different PLGA concentrations are shown in Fig. 7a. In general, the concentration of the released gentamicin was non-linearly increased from $t=0$ h to $t=10$ h in all the nanoparticles. The non-linear increase indicated that the gentamicin was continuously released by the nanoparticles in a complex pattern rather than linear fashion. Figure 7b shows a relationship between the concentration of the released gentamicin at the end of the 10-h period of testing and the PLGA concentrations used in the particle synthesis. It was found that the nanoparticles prepared at 0.0167 g/ml PLGA concentration had the largest gentamicin release rate. It was most likely due to a uniform porous structure and a great number of pores of the nanoparticles as shown in Fig. 4c. While the nanoparticles prepared at 0.0200 g/ml PLGA concentration showed the smallest average release rate possibly due to poorly uniform pores of the nanoparticles as shown in Fig. 4e.

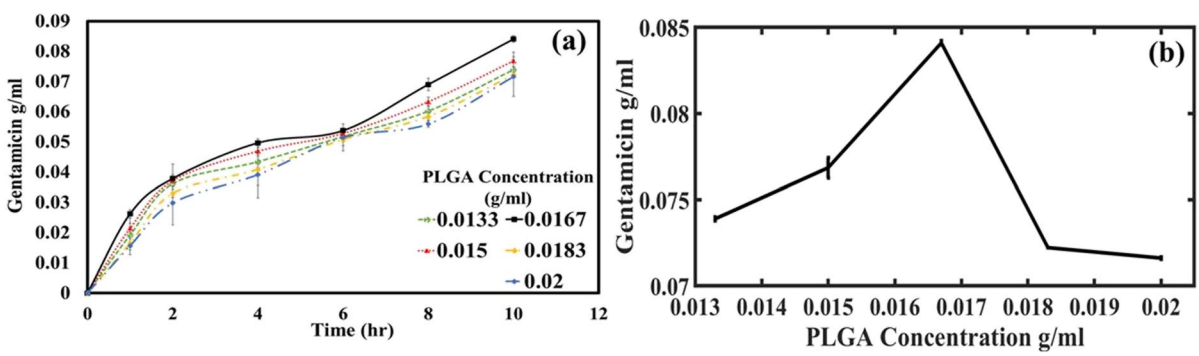

Fig. 7 **a** Represents the nanoparticles made at different PLGA concentrations (0.0133 g/ml, 0.0150 g/ml, 0.0167 g/ml, 0.0183 g/ml, and 0.0200 g/ml). **b** The concentration of gentamicin released from different PLGA nanoparticles made at different PLGA concentrations at 10 h. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle.

PLGA nanoparticle degradation

Nanoparticle degradation widely occurs in polymer-based nanoparticles and is commonly found in drug delivery system. The degradation rate is usually represented by the change in the weight ratio of the nanoparticles over time. In the PLGA nanoparticle degradation study, the calculated weight ratio was the weight of particles at time t relative to the initial weight of nanoparticles at time t_0. The degradation rate was studied for the nanoparticles obtained at different PLGA concentrations (0.0133 g/ml, 0.0150 g/ml, 0.0167 g/ml, 0.0183 g/ml, 0.0200 g/ml) used in particle synthesis. Figure 8 shows the weight ratio of the PLGA nanoparticles as a function of degradation time from $t = 0$ h to $t = 10$ h.

The weight ratio profiles of PLGA nanoparticles demonstrated a non-linear reduction that was more than 50% after 10 h for all the nanoparticles, suggesting that the degradation of nanoparticle was important in the release of gentamicin. The degradation rate profiles were different with a comparison of the nanoparticles prepared with different PLGA concentrations as shown in Fig. 8. The nanoparticles prepared at low PLGA concentrations (0.0133 g/ml, 0.0150 g/ml, and 0.0167 g/ml) showed a faster degradation rate than that obtained at high PLGA concentrations (0.0183 g/ml and 0.0200 g/ml), especially within the first 2 h. The particles made with 0.0133 g/ml PLGA were reduced to approximately 10% weight after 10 h, suggesting a nearly complete degradation of the particles.

Gentamicin release and nanoparticle degradation mechanism

Nanoparticle degradation and gentamicin release could be accounted for most of the lost weight in the gentamicin-loaded PLGA nanoparticles. The non-linear profiles of gentamicin release as shown in Fig. 7a suggested that the release process was governed by multiple rather than a single mechanism. There are primarily four release mechanisms in drug delivery systems: diffusion through polymers, osmotic pumping, diffusion through water-filled pores, degradation of nanoparticles (Pakulska et al. 2016).

First, in a polymer-based drug delivery system, diffusion through polymers is the most common release mechanism throughout the whole release process. Concentration gradient and temperature are the key parameters. Even though this mechanism exists throughout the release process, the release rate caused by diffusion through polymers can be small. Second, osmotic pumping is originated due to water absorption leading to driving the release of drugs. Drug release through osmotic pumping is affected by the pore channel length. Previous research has shown that the nanoparticles that have small pore size and long channel length are likely able to release drug through osmotic pumping (Pakulska et al. 2016). Third, the degradation of nanoparticles widely occurs in polymer-based nanoparticles. The particles with large diameter are likely to degrade and the drug is released. Forth, diffusion through water-filled pores highly is dependent with the pore structure of nanoparticles. Nanoparticle diameter and pore size are

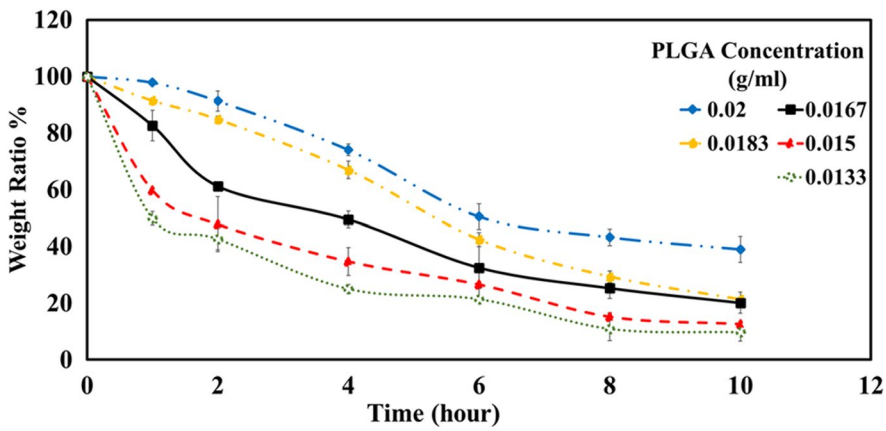

Fig. 8 The weight ratio profiles of the PLGA nanoparticles prepared at different PLGA concentrations. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle

the primary factors of diffusion through water-filled pores. This can be the primary mechanism at the beginning of release process. In the current study, the degradation and release processes of the PLGA nanoparticles were most likely governed by multiple mechanisms. Figure 4 shows significant differences in the porous structures of the PLGA nanoparticles at different PLGA concentration. The different porous structures resulted in different gentamicin release and particle degradation profiles. In the first few hours, the nanoparticle degradation was slow and hence the lost weight of the nanoparticle was mainly due to the release of gentamicin. In general, within the first few hours, the release of gentamicin was highly associated with the nanoparticle structure. The nanoparticles that were prepared at low PLGA concentrations (0.0133 g/ml and 0.0150 g/ml) had large pore sizes (Fig. 4a–b). The concentration gradient in gentamicin concentration between inner particles and the outer liquid solution was significant at the beginning of the release. Therefore, the release of gentamicin within the first 2 h was most likely controlled by the diffusion through water-filled pores, resulting in a fast release rate for the first 2 h (Fig. 7a). After that, gentamicin release can be attributed to a combination of nanoparticle degradation and other release mechanisms between 4 and 7 h. The concentration gradient of gentamicin then was reduced as a result of initial gentamicin release. Therefore, a decrease in the release rate was followed within 2 to 4 h (Fig. 7a). From 4 to 8 h, the release rate may be based on gentamicin diffusion combined with nanoparticle degradation. As the gentamicin concentration gradient was decreased, the gentamicin diffusion through the polymer competed with the diffusion through water-filled pores. In the last few hours, the nanoparticle degradation became significantly dominant in the release of the remaining gentamicin.

In the particles made at a medium concentration of PLGA (0.0167 g/ml), the release rate was unique compared to other nanoparticles. As shown in Fig. 4c, the nanoparticle had a uniformly porous structure, suggesting a good probability of similarly spherical shape. According to the DLS experiments, the nanoparticles synthesized with 0.0167 g/ml PLGA had the smallest average particle size. The release profile within the first 4 h shown in Fig. 7a was smooth, suggesting that the main release mechanism was likely diffusion through polymers according to a classical Higuchi model developed for a diffusion process. The Higuchi model for diffusion can be used in the release profiles when the polymer nanoparticles are small and the drug solubility in the outer aqueous phase is low. The release rate was decreased while the PLGA nanoparticle degradation rate was increased between 4 and 6 h as shown in Fig. 7a. The release rate was decreased due to a decrease in the gentamicin concentration gradient. After 6 h, the main release mechanism most likely became the nanoparticle degradation.

The nanoparticles synthesized with high PLGA concentrations (0.0183 g/ml and 0.0200 g/ml) had a large particle diameter and small pore size that most likely resulted in long pore channels as shown in Fig. 4d. Within the first 2 h, the diffusion through water-filled pores was likely the main release mechanism due to high concentration gradient as shown in Fig. 7a. After 2 h, the release process might have been dominated by osmotic pumping due to strong water absorption through the long pore channels. At the meantime, the nanoparticle degradation was slowly taking place and became significant after 5 h. The large size of the particles might promote the degradation of the particles.

Antibacterial activity

A dish diffusion method was used to investigate the antibacterial activity of nanoparticles as shown in Fig. 9. The yellow area in each petri dish was where the growing *E. coli* was spreading. The 5 circle wells were *E. coli*-free, which was bacterial inhibition zones. The size of the wells varied in different nanoparticle, which was the measure of antibacterial activity. The antibacterial activities were found in the gentamicin-loaded PLGA nanoparticles as shown in Fig. 9b–f, while there was no antibacterial activity found in PLGA nanoparticles without gentamicin loaded as shown in Fig. 9a. A 0.1 g/ml gentamicin only (no PLGA nanoparticles) solution showed nearly no *E. coli* growth as expected.

The antibacterial activities of PLGA nanoparticles and pure gentamicin were evaluated by measuring the diameters of bacterial inhibition zones and the results are shown in Fig. 10.

The diameter of bacterial inhabitation zone was found more than 13 mm for all of the PLGA nanoparticles, suggesting effective antibacterial properties

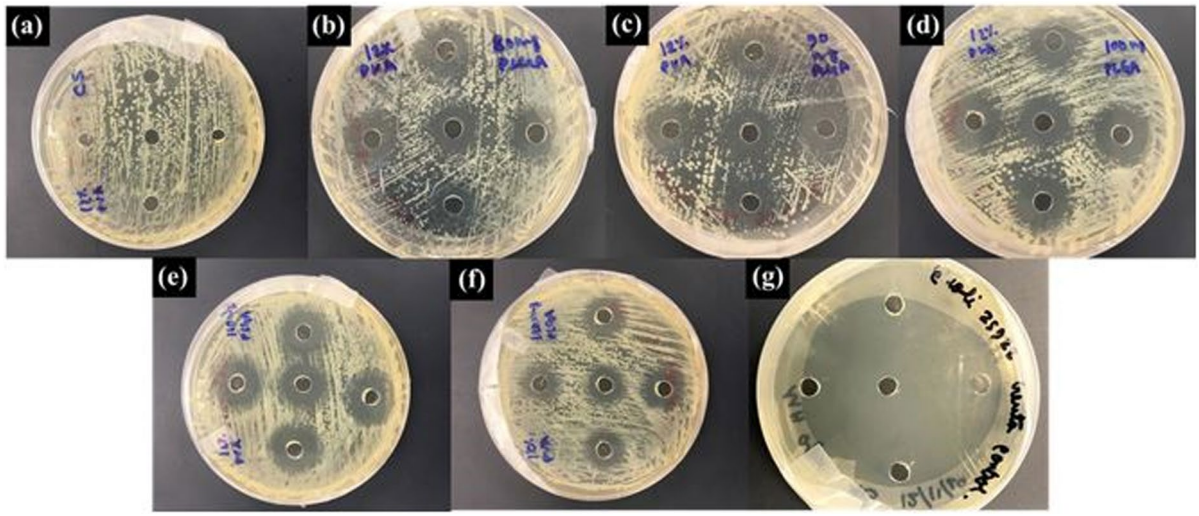

Fig. 9 Photographs of different PLGA nanoparticles' antibacterial activity. **a** Controlled PLGA without loading gentamicin; **b–f** represents the nanoparticles at different PLGA concentrations (0.0133 g/ml, 0.0150 g/ml, 0.0167 g/ml, 0.0183 g/ml, and 0.0200 g/ml); **g** pure gentamicin solution. The photographs are reported with five sample measurements for each nanoparticle

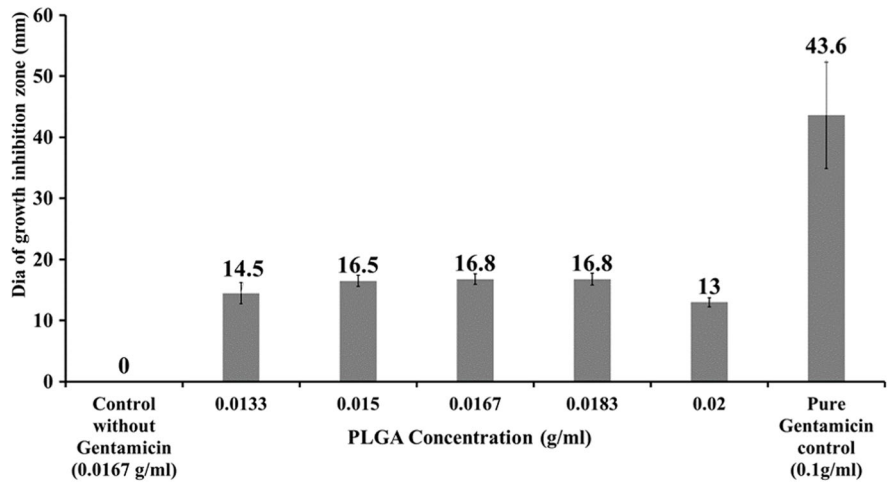

Fig. 10 Inhibition zone diameters of various PLGA nanoparticles. The data are reported with five sample measurements for each nanoparticle. The error bars are calculated with the five measurements for each nanoparticle

of the gentamicin-loaded PLGA nanoparticles. The nanoparticles that were prepared with 0.0167 g/ml and 0.0183 g/ml PLGA concentrations showed largest diameters (16.8 mm) of inhibition zones, suggesting the most effective antibacterial properties. The effectiveness of antibacterial properties was primarily a result of porous structures of the gentamicin-loaded PLGA nanoparticles. In addition, the uniformity and fineness of the porous structures in the nanoparticles were able to promote the antibacterial activity, which was demonstrated by the nanoparticles made at 0.0167 g/ml PLGA concentration. In addition, it was expected that the gentamicin-only solution demonstrated the largest inhabitation diameter as shown in Fig. 10, confirming effective antibacterial properties of gentamicin used as an antibiotic.

Conclusion

This paper demonstrates a double emulsion method of fabricating antibiotic-encapsulated PLGA nanoparticles that shows uniform porous nanostructures, time-dependent drug release and particle

degradation profiles, and good antimicrobial performance. PLGA nanoparticles were prepared and loaded with gentamicin via a double emulsion evaporation method with different PVA (surfactant) and PLGA concentrations. The gentamicin-loaded PLGA nanoparticles were generally spherical and porous whereas the PVA and PLGA concentrations were critical factors in determining the particle structure. The possibility of spherical nanoparticle formation and the particle yield was primarily increased with an increase in PVA concentrations. The porous structure was significantly dependent on the PLGA concentrations. The nanoparticles showed various release properties determined by multiple release mechanisms. Diffusion through water-filled pores occurred most likely at the beginning of the release process. Nanoparticle degradation became the dominant mechanism after a few hours. In addition, diffusion through polymers and osmotic pumping occurred after a few hours during the release process. All the gentamicin-loaded PLGA nanoparticles demonstrated effective antibacterial activities. The particle morphology demonstrated a significant effect on nanoparticle degradation and gentamicin release profiles, as well as antibacterial activities. The nanoparticles made at over 8% PVA concentration have spherical shape and high yield percentage. Nanoparticles synthesized with different PLGA concentrations show different release profiles and size distribution, but all of them have antibacterial activities to some degree. Therefore, the gentamicin-loaded PLGA nanoparticles could be tuned using the double emulsion evaporation method with different parameters including PVA (surfactant) concentration and PLGA concentration, resulting in effective antibacterial activity.

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Declarations

Conflict of interest The authors declare no competing interests.

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