J Mater Sci: Mater Med
DOI 10.1007/s10856-013-5131-y

In-vitro anticancer and antimicrobial activities of PLGA/silver nanofiber composites prepared by electrospinning

Fahad N. Almajhdi · H. Fouad · Khalil Abdelrazek Khalil · Hanem M. Awad · Sahar H. S. Mohamed · T. Elsarnagawy · Ahmed M. Albarrag · Fawzi F. Al-Jassir · Hany S. Abdo

Received: 18 May 2013 / Accepted: 23 December 2013

Abstract In the present work, a series of 0, 1 and 7 wt% silver nano-particles (Ag NPs) incorporated poly lactic-co-glycolic acid (PLGA) nano-fibers were synthesized by the electrospinning process. The PLGA/Ag nano-fibers sheets were characterized using SEM, TEM and DSC analyses. The three synthesized PLGA/silver nano-fiber composites were screened for anticancer activity against liver cancer cell line using MTT and LDH assays. The anticancer activity of PLGA nano-fibers showed a remarkable improvement due to increasing the concentration of the Ag NPs. In addition to the given result, PLGA nano-fibers did not show any cytotoxic effect. However, PLGA nano-fibers that contain 1 % nano silver showed anticancer activity of 8.8 %, through increasing the concentration of the nano silver to 7 % onto PLGA nano-fibers, the anticancer activity was enhanced to a 67.6 %. Furthermore, the anti-bacterial activities of these three nano-fibers, against the five bacteria strains namely; *E.coli* o157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566 using the disc diffusion method, were evaluated. Sample with an enhanced inhibitory effect was PLGA/Ag NPs (7 %) which inhibited all strains (inhibition zone diameter 10 mm); PLGA/Ag NPs (1 %) sample inhibited only one strain (*B. cereus*) with zone diameter 8 mm. The PLGA nano-fiber sample has not shown any antimicrobial activity. Based on the anticancer as well as the antimicrobial results in this study, it can be postulated that: PLGA nanofibers containing 7 % nano silver are suitable as anticancer- and antibiotic-drug delivery systems, as they will increase the anticancer as well as the antibiotic drug potency without cytotoxicity effect on the normal cells. These findings also suggest that Ag NPs, of the size (5–10 nm) evaluated in the present study, are appropriate for therapeutic application from a safety standpoint.

F. N. Almajhdi
Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

H. Fouad (✉)
Biomedical Engineering Department, Faculty of Engineering, Helwan University, Helwan, Egypt
e-mail: menhfefnew@hotmail.com

K. A. Khalil · H. S. Abdo
Mechanical Engineering Department, College of Engineering, King Saud University, P.O. Box 800, Riyadh 11421, Saudi Arabia

H. M. Awad · S. H. S. Mohamed
National Research Centre, Dokki, Cairo 12622, Egypt

T. Elsarnagawy
Faculty of Engineering, Prince Sultan University, Riyadh, Saudi Arabia

A. M. Albarrag
Department of Pathology, College of Medicine and the University Hospitals, King Saud University, Riyadh, Saudi Arabia

F. F. Al-Jassir
FRCSC, College of Medicine, Orthopedic Surgery Research Chair King Saud University, Riyadh, Saudi Arabia

1 Introduction

Controlled drug delivery systems have gained much attention in the last few decades. This is due to their advantages compared with the conventional dosage forms such as improving therapeutic efficacy and reducing toxicity by delivering them at a controlled rate. Polymers

Published online: 31 December 2013

contain metal nano-particles which are used as antimicrobial or drug delivery systems, have been captured attention, because of their novelty in being long-lasting biocidal materials with high temperature stability and low volatility [1]. The large increase in the number and occurrence of antibiotic-resistant bacterial strains has prompted a renewed interest in the use of metallic nanoparticles (NPs) as antibacterial agents [2].

Nowadays, the introduction of silver nano-particles (Ag NPs)-based antimicrobial polymers represents a great challenge for both academic world and industry [3, 4]. The silver-based thermoplastic polymer composites combine the excellent high temperature processibility of the thermoplastics with the inherent antimicrobial property of the silver. Silver ions are significant antimicrobials by virtue of their antiseptic properties with only few bacteria being intrinsically resistant to this metal [5]. Silver is well known being a significant resource for topical therapy because of its beneficial antimicrobial properties in medical devices such as cannulae and catheters [6, 7].

Recently, ultrafine polymer fibers were explored as new devices for drug delivery. The main advantages of the fibrous carriers are that they offer site-specific delivery of drugs to the body. In addition, more than one drug can be encapsulated directly into the fibers. Due to the high surface area and porous structure of the electrospun fibers, they have been applied in many fields such as medicine, biosensors, sensitized solar cells, tissue engineering, photonics, nano-composites, catalysts, antimicrobial materials and membranes [8–11]. There are several techniques used for the production of polymeric ultrafine fibers (UFs), including electrospinning technique [12–14]. This technique has evinced more interest in recent years because of its versatility and potential for applications in various fields. It is popular for its simple process operation, high performance in nanofiber fabrication and low in cost.

Poly(D,L-lactic-*co*-glycolic acid) polymers (PLGA) are ones of the most commonly used for preparing eroding drug-release devices. PLGA is degraded by metabolic pathways and therefore does not require surgical removal after completion of drug release to be injected, implanted or inserted. The formulations based on PLGA microparticles and implants may provide long-term sustained release. They are useful for local treatments, such as infections in bones and soft tissues, or systemic delivery of labile drugs, such as peptides, hormones or vaccines. Due to these properties; PLGA is considered as one of the most popular biodegradable polymers approved by the US Food and Drug Administration (FDA) [15]. Therefore, PLGA has been widely used in drug-delivery applications and tissue engineering scaffolds [16–21].

In our previous work, we reported a new class of poly lactic-co-glycolic acid (PLGA) nanofibers containing Ag NPs as electrospun composite nanofiber-based drug delivery systems [17]. However, the anticancer and antibacterial efficacies of those PLGA/Ag NPs nanofibers have not been explored. Therefore, in this study, with the continuation of our previous work, we evaluated the anticancer activity of PLGA nanofibers containing different Ag NPs percentages against liver carcinoma cell line using 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. Finally, the antimicrobial efficacy of PLGA/Ag NPs against different types of bacteria was evaluated as well.

2 Materials and methods

2.1 Materials

The biodegradable polymer that is used in the present study is poly (D,L-lactide-*co*-glycolide) (PLGA) copolymer with L/G ratio of (75/25 mol/mol). The PLGA that purchased from NaBond Company, China has inherent viscosity of 0.59 (dl/g), weight-average molecular weight of 70,000 and density of 1.25–1.3 g/cm^3. Tetrahydrofuran (THF), dimethylformamide (DMF) solvents and phosphate buffer saline (PBS) pellets that used in the experiments were purchased from Sigma Aldrich.

Roswell Park Memorial Institute (RPMI) 1640 medium and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and fetal calf serum (FCS) were purchased from Gibco, United Kingdom. Dimethyl sulfoxide (DMSO) and methanol were of HPLC grade and all other reagents and chemicals were of analytical reagent grade.

The microorganisms used for the experiment are from the Microbiological Resources Center (Cairo MIRCEN) Faculty of Agriculture, Ain Shams University: *Escherichia coli* o157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566.

2.2 Electrospinning of PLGA and PLGA/Ag NPs nanofibers

The electrospun PLGA and PLGA/Ag NPs nanofibers, containing 1 and 7 % Ag NPs, were prepared by electrospinning of a PLGA solution (10 wt%) in DMF/THF (90 wt%) and characterized using SEM, TEM and DSC analyses as described in our previous work [17]. The nanofibers were collected on a rotating target. A voltage ranges from 10 to 20 kV was applied to the collecting target by a high voltage power supply, and the flow rate of the solution ranges from 0.1 to 0.15 mL/h. Regarding PLGA/Ag NPs nanofibers fabrication, $AgNO_3$ at different

ratios are dissolved in the DMF/THF, and then 0.01 gm of polyethylene glycol was added as a stabilizer and reduction agent. This solution was stirred for 30 min before analysis using UV spectra, then PLGA was added to the solution. The solution containing silver salt was stirred for 24 h at room temperature. The reduction of Ag^{+} ions into elemental Ag^{0} in the form of NPs, takes place through a series of steps: primary nucleation, secondary nucleation, or crystal growth via diffusion mechanism to obtain primary particles, and agglomeration of some adjacent primary particles into aggregated clusters (i.e., secondary particles) [22]. The electrospun PLGA and PLGA/Ag NPs nanofibers could be readily peeled off from the aluminum foil, and the obtained nano-fibrous were stored at room temperature for 7 days before the subsequent characterizations.

2.3 Characterization methods

2.3.1 Morphology examination

Scanning electron microscopy (FE-SEM-JEOL GSM-6610LV) was used for characterizing the surface morphology of PLGA; PLGA/Ag NPs nanofibers. The specimens were coated with platinum and examined at an accelerated voltage of 10 kV.

2.3.2 Nanofibers porosity

For calculating the PLGA and PLGA/Ag NPs apparent porosity, the apparent density of the electrospun nanofibers was firstly calculated. The nanofibers sheet density was estimated by the measurement of volume and mass of samples (8 samples at least) according to Eq. (1) [16].

$$\text{Apparent density } (\rho) = \frac{\text{Mass of nanofiber sheet (g)}}{\text{Volume of sheet (cm}^3\text{)}} \left(\text{g/cm}^3\right) \quad (1)$$

The apparent porosity of electrospun scaffolds was then estimated using Eq. (2).

$$\text{Apparent Porosity } (\%) = \left[1 - \frac{\rho}{\rho_0}\right] \times 100\ \% \quad (2)$$

where ρ is the estimated apparent density of the electrospun sheet and ρ_0 is the bulk density of the PLGA (1.25–1.3 g/cm^3).

Where the estimated apparent density of the elctrospun is and the bulk density of the PLGA (1.25–1.3 g/cm^3) is.

2.3.3 Thermogravimetric analysis (TGA)

Thermogravimetric analysis of PLGA; PLGA/Ag NPs nano-fibers was conducted using TA instrument (Q500 TGA, US). The nano-fibers sheets were kept under vacuum prior to testing. The specimen was heated starting from 25 to 400 °C at heating rate of 15 C/min under nitrogen flow with obtaining the weight loss.

2.3.4 Thermal properties (DSC)

Thermal characteristics of the PLGA and PLGA/Ag NPs nanofibers were evaluated using differential scanning calorimetry (DSC). Differential scanning calorimeter (Shimadzu -60) was used to measure the glass transation temperature (Tg) of PLGA and PLGA/Ag NPs nanofibers. PLGA; PLGA/Ag NPs nanofibers sample ($\approx$5 mg) is sealed in an aluminum pan and heated starting from 25 to 80 °C at rate of 2 °C/min, then cooled down to 20 °C.

2.3.5 Antibacterial assay using the disc diffusion method

Screening of different samples (3 discs samples in 5 mm diameter) was tested by disc diffusion method. Each sample (5 mm in diameter) was inoculated on Tryptose soy agar supplemented with yeast extract (TSAYE) in a standard Petri dish from a 16 to 18 h culture grown in TSAYE broth inoculums and they were incubated at 37 °C. The concentration of bacteria inoculated in TSAYE was 2×10^6 cfu/ml. All experiments were performed in triplicate. The inhibition zone diameter was measured and expressed in millimeters.

2.3.6 In vitro anticancer activity

2.3.6.1 Cell culture Human liver carcinoma cell line (Hep-G2) was purchased from the American Type Culture Collection (Rockville, MD) and maintained in RPMI-1640 medium. Normal human amnion cell line (WISH) was kindly supplied by Applied Research Sector, VACSERA-Egypt and maintained in DMEM medium. Both media were supplemented with 10 % heat-inactivated FBS, 100 U/ml penicillin and 100 U/ml streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5 % CO_2. All experiments were conducted in triplicate ($n = 3$). All the values were represented as mean ± SD.

2.3.6.2 In vitro antitumor activities: MTT cytotoxicity assay The cytotoxicity activity against Hep-G2 and WISH human cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [23]. Cells were dispensed in a 96 well sterile microplate (5×10^4 cells/well), and incubated at 37 °C with a disc of 5 mm diameter of each tested sheet or doxorubicin (positive control) for 48 h in a serum free

medium prior to the MTT assay. After incubation, media and disks were carefully removed, 40 μL of MTT (5 mg/mL) was added to each well and then incubated for an additional 4 h. The purple formazan dye crystals was solubilized by the addition of 200 μL of acidified isopropanol. The absorbance was measured at 570 nm using a microplate ELISA reader (Biorad, USA). The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

2.3.6.3 LDH assay To determine the effect of each sheet on membrane permeability in both Hep-G2 and WISH cell lines, a LDH release assay was used [24]. The cells were seeded in 96-well culture plates at a density of 2×10^4 cells/well in 100 μL volume and allowed to grow for 18 h before treatment. After treatment, with disks of 0.5 cm diameter of each tested sheet, the plates were incubated for 48 h. Discs were removed. Then, the supernatant (40 μL) was transferred to a new 96 well to determine LDH release and 6 % triton X-100 (40 μL) was added to the original plate for determination of total LDH. An aliquot of 0.1 M potassium phosphate buffer (100 μL, pH 7.5) containing 4.6 mM pyruvic acid was mixed to the supernatant using repeated pipetting. Then, 0.1 M potassium phosphate buffer (100 μL, pH 7.5) containing 0.4 mg/mL reduced β-NADH was added to the wells. The kinetic changes were read for 1 min using ELISA microplate reader in absorbance at wavelength 340 nm. This procedure was repeated with 40 μL of the total cell lysate to determine total LDH. The percentage of LDH release was determined by dividing the LDH released into the media by the total LDH following cell lysis in the same well.

2.3.7 Statistical analysis

All the experiments were repeated at least three times, conducted in triplicate ($n = 3$) and error bars represent standard deviations (SD). All the values were represented as mean ± SD.

3 Results and discussion

3.1 Fabrication of PLGA/Ag NPs nanofibers

The electrospun nanofibers of PLGA and PLGA/Ag NPs were governed due to the presence Coulomb force between the charges on the jet surface, external electric field force, viscoelastic force of the solution, surface tension force, gravitational force, and the frictional force due to air drag. The electrospun nanofibers were collected on the rotating drum target as a nonwoven fabric with superior characteristics such as large surface area-to-volume ratio high porosity and acceptable pore size. The electrospun samples were dried for one day at room temperature to remove the solvent.

3.2 Morphology of PLGA nanofibers

The cell adhesion and proliferation on the nanofibers scaffolds were affected by scaffold properties; such as surface morphology, porosity and chemical composition. The electrospun PLGA and PLGA/Ag NPs (1 and 7 %) nanofibers surface morphology was examined using (JEOL GSM-6610LV) SEM. The SEM results (Fig. 1) indicated that the electrospun PLGA and PLGA and PLGA/Ag NPs nanofibers have a smooth morphology. All fibers exhibited uniform diameters with several micrometers in length. It was also observed that most of the as-spun nanofibers are round and uniform over its length. As shown in Fig. 1a, b, pure PLGA and PLGA/Ag NPs (1 %) nanofibers produce smooth and bead-free nanofibers. Observable beads can be noticed with high silver nitrate contents (Fig. 1c). This could be attributed to high conductivity and/or low viscosity of the solution due to the changes of silver content [17]. Many experiments have shown that an optimum viscosity for each polymer solution is required to produce fibers without beads [25, 26]. The diameter of the electrospun nanofiber increases with increasing the viscosity [27, 28].

Figure 2 shows the TEM image of PLGA/Ag NPs (7 %) nanofibers. This figure indicates that the Ag NPs (with nearly spherical shape and equal size) lie in/on the nanofibers. The diameter of nanofibers is within the range of 100–200 nm, while the size of Ag NPs was within 5–10 nm range.

3.3 Nanofibers porosity

In tissue engineering applications, the porosity of the scaffold is an essential parameter for the success of cell culture experiments. Highly porous scaffolds afford cell attachment, proliferation, differentiation and sufficient transport for nutrients and waste removal. Therefore, it is important to fabricate the 2D or 3D scaffolds with sufficient and acceptable porosity. In the present work the porous scaffolds apparent density and porosity of electrospun PLGA nanofibers and PLGA/Ag NPs sheets were calculated using Eqs. (1) and (2). The density and porosity of PLGA nanofibers sheet fabricated with the electrospinning technique were found to be 0.4065 g/cm^3 and 63 ± 3 %, respectively. The calculated apparent density of PLGA/Ag NPs (1 and 7 %) nanofibers sheets were found to be 0.48, 0.53 g/cm^3 and porosity of 61 ± 4 %, 58 ± 3 % respectively. These highly porous scaffolds were beneficial for the adherence and proliferation of the cells. Similar values of PLGA nanofibers porosity (64–70 %) have been obtained when using the electrospinning technique for nanofibers fabrication [16].

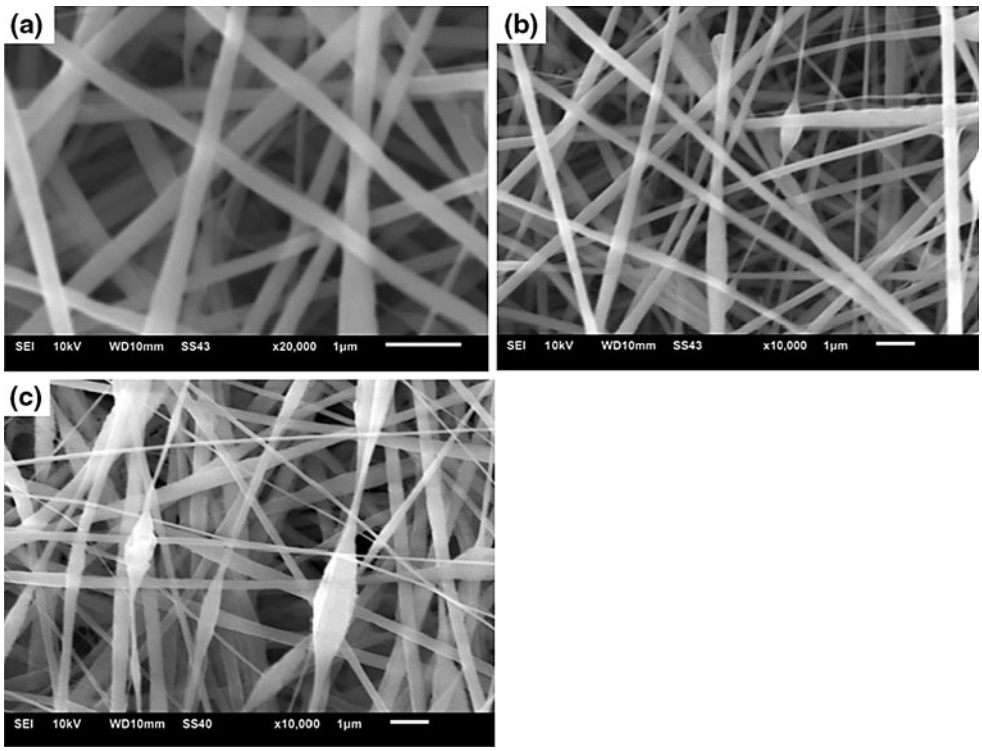

Fig. 1 SEM images of **a** PLGA nanofibers and **b** PLGA nanofibers containing 1 % Ag NPs and **c** 7 % Ag NPs

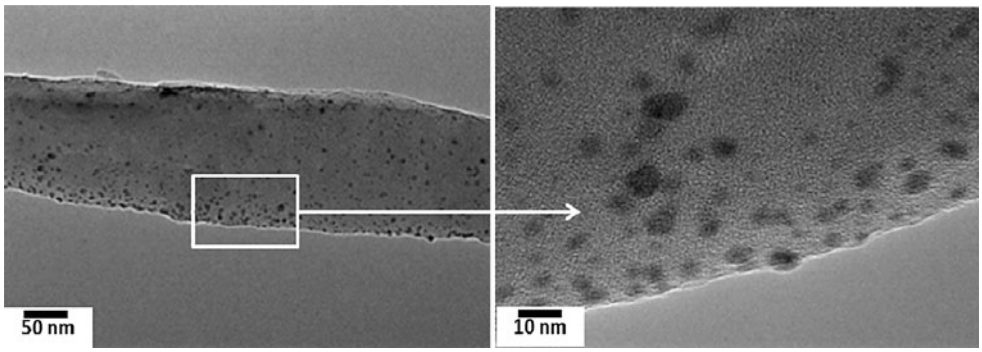

Fig. 2 TEM images of PLGA nanofibers containing 7 % Ag NPs

3.4 TGA and DSC analysis

The TGA of PLGA and PLGA/Ag NPs (7 %) nanofibrous sheets at heating rate of 15 °C and at temperature up to 400 °C is shown in Fig. 3. The results show that there is no weight loss up to 250 °C. The weight loss mainly occurred in the temperature range from 260 to 360 °C with negligible change at temperature higher than 360 °C. This weight loss indicates thermal decomposition or evaporation in the material. From this figure, it is clear that specimens' weight loss occurred mainly due to the combustion of organic PLGA matrix. The results of Fig. 3 also indicated that the degradation temperature of the PLGA/Ag NPs decreased and the weight loss increased with the presence (increasing) of Ag NPs. This means that the thermal

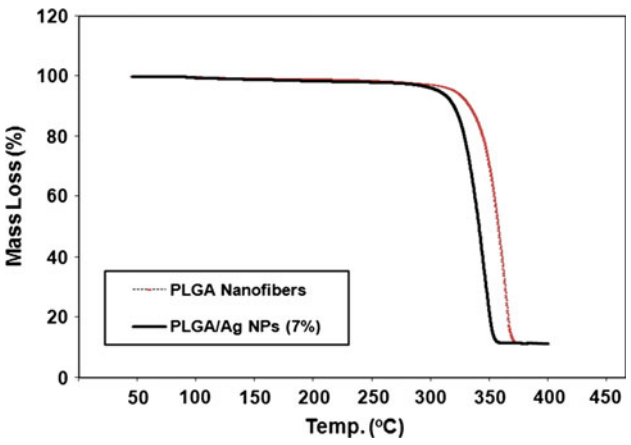

Fig. 3 TGA of the PLGA and PLGA/Ag NPs (7 %) nanofibers

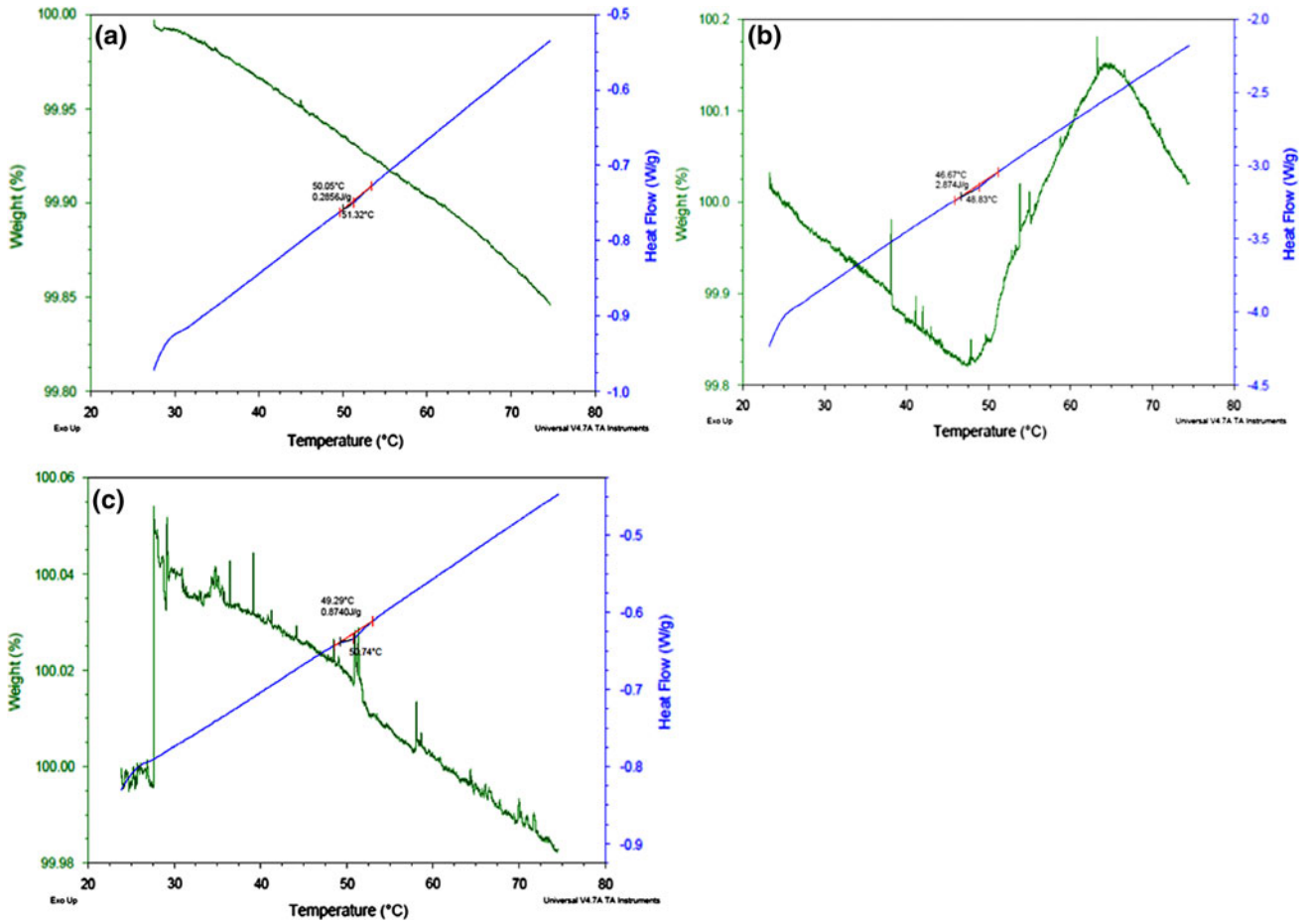

Fig. 4 DSC of **a** PLGA, **b** PLGA/Ag NPs (1 %) and **c** PLGA/Ag NPs (7 %) nanofibers

stability of the PLGA nanofibrous sheets were decreased due to the presence of Ag NPs in the PLGA nanofibers. These results can be attributed to the high thermal conductivity of Ag NPs compared to the polymer fibers.

The DSC thermograph of PLGA and PLGA/Ag NPs (1 and 7 %) scaffolds that were obtained at temperature ranges from 25 to 80 °C are shown in Fig. 4. The DSC cures results showed that all of the tested materials have low glass transition temperature (50 °C, higher than the physiological human body temperature). Pervious DSC results [17] indicated that there is no melting point for PLGA and PLGA/Ag NPs (1 and 7 %) nanofibers, indicating that the polymer is essentially amorphous. The results of Fig. 4 also showed that the T_g of PLGA nanofibers slightly decreased due to the addition of Ag NPs.

3.5 Antibacterial activity

Several polymers have been used to stabilize Ag NPs, including polyethyleneimine [29], polyallylamine [30], poly(vinylpyrrolidone) [31], and chitosan [32]. The nucleophilic character of these polymers may be sufficient for binding with the metal particles by donating electrons [33]. The FDA-approved, biodegradable and biocompatible polymer PLGA has been chosen in this study because of its hydrolysable ester bonds, that are subjected to the nucleophilic interaction with Ag NPs [34]. The antibacterial activity is a very important characteristic of any material that is intended for biomedical applications. Therefore, PLGA nanofibers as well as PLGA/Ag NPs (1 %) and PLGA/Ag NPs (7 %) nanofibers were investigated for their antibacterial activities against three Gram-positive bacteria (*B. cereus* EMCC 1080, *S. aureus* ATCC 13565 and *L. monocytogenes* EMCC 1875) and two Gram-negative bacteria (*E.coli* o157:H7 ATCC 51659 and *S. typhimurium* ATCC25566).

PLGA nanofibers specimens (0, 1 and 7 % Ag NPs) showed various degrees of inhibition against the five bacteria strains using the disc diffusion method as presented in Fig. 5. Sample with an enhanced inhibitory effect was PLGA/Ag NPs (7 %) which inhibited all the five strains with inhibition zone diameter of 10 ± 1.27 mm; PLGA/Ag

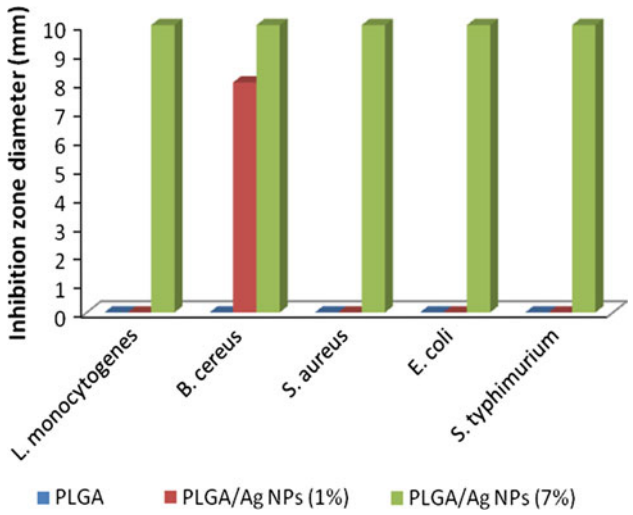

Fig. 5 Antibacterial activity of the three PLGA nanofibers sheets, containing different percentages of silver nanoparticles, against the five bacterial strains. The diameter of each sample disc (5 mm) is included

NPs (1 %) sample inhibited only one strain (*B. cereus*) with zone diameter of 8 ± 0.14 mm. The PLGA nanofiber sample has not shown any antimicrobial activity. These results reveal that the antibacterial activity of the PLGA nanofibers increased by increasing the concentration of the Ag NPs incorporated into the polymer. These results are inlined with the previously reported data. One of the studies, in vitro microbiological studies of electrospun PLGA UFs, using *S. aureus*, *Ps. aeruginosa* and a methicillinresistant *S. aureus*, shows fast bacterial colonization of UFs and formation of a dense biofilm [35]. In another study, the results from in vitro assay demonstrated that 2 % Ag NP/PLGA coating effectively prevented bacterial adherence and biofilm formation, of Gram-positive *S. aureus* Mu50 or Gram-negative *P. aeruginosa* PAO-1 on stainless steel alloy implants [36]. However, the lower percentages of Ag NPs/PLGA than 2 % did not show any significant antibacterial activities. *Bacillus* species are found to be more sensitive than *E. coli* to the Ag NPs [37]. Using silver nanocrystals encapsulated in mesoporous silica NPs, *B. subtilis* showed higher sensitivity compared to *E. coli*, [38]. Moreover, *Bacillus* was also showed the highest susceptibility to silver and copper NPs [39]. Variations in the degree of antimicrobial activity of Ag NPs have been reported. Most of the studies found that smaller particles have large surface area and are more toxic to bacteria. Other factors like morphology of NPs, physical and chemical properties also have strong impact on antimicrobial activity.

The findings of this study can be explained by the fact that, silver prevents cell division and transcription by binding to and disrupting multiple components of bacterial structure and metabolism, including cellular transport, essential enzyme systems such as the respiratory cytochromes, and synthesis of cell wall components, DNA and RNA [36]. Ionic forms of silver such as silver nitrate ($AgNO_3$) and silver sulfate (Ag_2SO_4) have been used to provide protection against bacterial infections. However, despite its effective short-term antibacterial activity, inadequate local retention and severe cytotoxic effects of ionic silver (Ag^+) have made it undesirable for continually preventing bacterial colonization on the implants. Recent reports have shown that 20–25 nm Ag NPs effectively inhibit microorganisms without causing significant cytotoxicity, and that 10–20 nm Ag NPs are nontoxic in mice and guinea pigs when administered by the oral, ocular and dermal routes [40]. In this study, the Ag NPs size was found to be 5–10 nm, which may be even better as it shows inhibitory activity, at lower concentration (1 % AgNPs) than that reported previously, against *B. cereus*.

3.6 In vitro anti-cancer activity

The biocompatible polymer PLGA nanofibrous has been chosen in this study as anticancer drug delivery system, because PLGA degradation is based on hydrolytic splitting of the polymer backbone into oligomers and release of lactic acid and glycolic acid, two byproducts of various metabolic pathways in the body under normal physiological conditions [41]. Therefore, it does not require surgical removal after completion of drug release. Furthermore, the incorporation of the biocompatible Ag NPs dispersed in a PLGA polymer matrix may represent an important system in the field of medicine, nanomedicine, pharmacy and controlled drug delivery, and in particular to overcome resistance to antibiotics. Although biodegradable PLGA nanofibers containing Ag NPs polymeric matrix scaffolds have proved antimicrobial activity [41], no studies have been more extensively investigated with regard to their potential quality as anticancer agents.

In this study, PLGA nanofibrous, PLGA/Ag NPs 1 and 7 % nanofiberous were examined in vitro for their anticancer activities against Hep-G2 human liver cancer cell line and their cytotoxic activities against normal human amnion cell line (WISH) using MTT assay. This assay aims to measure the percentage of the intact cells compared to the control as well as LDH assay to measure the cellular membrane permeabilization (rupture) and severe irreversible cell damage (Fig. 6). The results show that, the anticancer activity of PLGA nano fibrous increased by increasing the concentration of the coated Ag NPs. PLGA nanofibers did not show any cytotoxic effect. However, PLGA nanofibers containing 1 % Ag NPs showed anticancer activity of 8.8 %, by increasing the concentration of the Ag NPs to 7 % onto PLGA nanofibers the anticancer activity enhanced to 67.6 %.

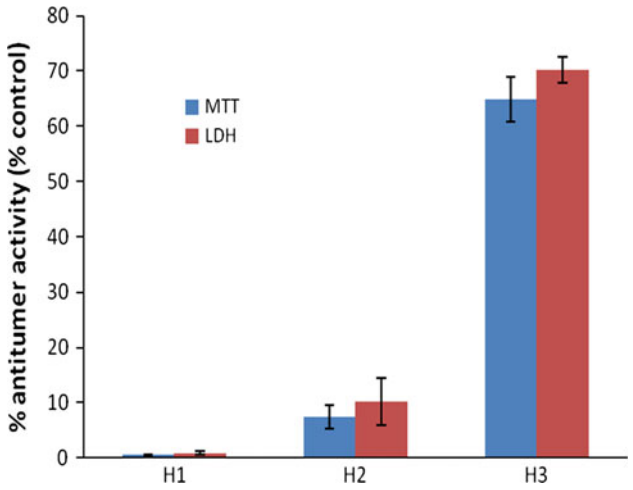

Fig. 6 Anticancer activities of three PLGA nanofibers sheets against liver carcinoma cell line (HepG-2) using both MTT and LDH assays. *H1* PLGA nanofibers alone, *H2* PLGA/Ag NPs 1 % and *H3* PLGA/Ag NPs 7 %

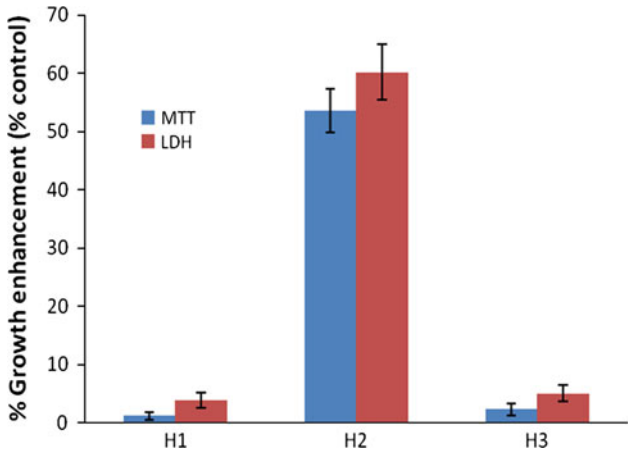

Fig. 7 Growth enhancement activities of three PLGA nanofibers sheets on human normal cell line (WISH) using both MTT and LDH assays. *H1* PLGA nanofibers alone, *H2* PLGA/Ag NPs 1 % and *H3* PLGA/Ag NPs 7 %

All the investigated PLGA nanofibers sheets did not show any toxic activity against the human normal cells (WISH). In the contrary, they all cause cytoprotective activities and growth enhancement ranging from 2.5 to 56.7 %, (Fig. 7). PLGA nanofibers showed only very low growth enhancement effect of 2.5 %. However, PLGA nanofibers containing 1 % Ag NPs showed growth enhancement effect of 56.9 % and by increasing the concentration of the Ag NPs to 7 % onto PLGA nanofibers, the growth enhancement decreased again to 3.7 %. There is no clear explanation for these findings. It could be the addition of Ag NPs at low percentage (1 %) that changes the surface properties of the PLGA nanofibers which enhanced the adherence of cells. The calculated apparent density and porosity of PLGA/Ag NPs nanofibers sheets were found were increased compared to PLGA nanofibers [17]. These highly porous scaffolds are also beneficial for the adherence and proliferation of the cells. The PLGA/Ag NPs 7 % are showing a decrease in growth enhancement as a result of cytotoxicity of Ag NPS to the cells.

The anticancer activity of PLGA/Ag NPS (7 %) may be attributed to the fact that Ag NPs, induce toxicity predominately through oxidative stress, by the generation of reactive oxygen species (ROS), which have specific effects in cells, including oxidative damage to DNA, protein and lipid [42] this may also explain the decrease in growth inhancment of PLGA/Ag NPS (7 %) against the human normal cells.

4 Conclusion

Based on the preliminary anticancer as well as the antimicrobial results in this study, it can be postulated that PLGA nanofibers containing 7 % Ag NPs may be suitable as anticancer- and antibiotic-drug delivery systems, as they may increase the anticancer as well as the antibiotic drug potency without cytotoxicity effect on the normal cells. These findings suggest also that Ag NPs, of the size (5–10 nm) evaluated in the present study, may be appropriate for therapeutic application from a safety standpoint. However, further studies are required to evaluate the contribution of these NPs to the specific health benefits in biological systems, in vitro as well as in vivo.

Acknowledgments The authors gratefully acknowledge funding from NPST at King Saud University Project No. (09-BIO676-02).

References

1. Kumar R, Münstedt H. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials. 2005;26:2081–8.
2. Stobie N, Duffy B, McCormack DE, Colreavy J, Hidalgo M, McHale P, Hinder SJ. Prevention of *Staphylococcus epidermidis* biofilm formation using a low-temperature processed silver-doped phenyltriethoxysilane sol–gel coating. Biomaterials. 2008; 29:963–9.
3. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents. 2009;34: 103–10.
4. Fortunati E, Mattioli S, Visai L, Imbriani M, Fierro JLG, Kenny JM, Armentano I. Combined effects of Ag nanoparticles and oxygen plasma treatment on PLGA morphological, chemical, and antibacterial properties. Biomacromolecules. 2013;14:626–36.
5. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med. 2007;3:95–101.

6. Gilchrist T, Healy DM, Drake C. Controlled silver-releasing polymers and their potential for urinary tract infection control. Biomaterials. 1991;12:76–8.
7. Joyce-Wohrmann RM, Hentschel T, Munstedt HT. Thermoplastic silver-filled polyurethanes for antimicrobial catheters. Adv Eng Mater. 2000;2:380–6.
8. Kenawy ER, Bowlin GL, Mansfield K, Layman J, Simpson DG, Sanders EH, Wnek GE. Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. J Controlled Release. 2002;81:57–64.
9. Zong X, Kim K, Fang D, Ran S, Hsiao BS, Chu B. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. Polymer. 2002;43:4403–12.
10. Zeng J, Xu X, Chen X, Liang Q, Bian X, Yang L, Jing X. Biodegradable electrospun fibers for drug delivery. J Controlled Release. 2003;92:227–31.
11. Kenawy ER, Abdel-Hay FI, El-Newehy MH, Wnek GE. Controlled release of ketoprofen from electrospun poly (vinyl alcohol) nanofibers. Mater Sci Eng A. 2007;459:390–6.
12. Luong-Van E, Grøndahl L, Chua KN, Leong KW, Nurcombe V, Cool SM. Controlled release of heparin from poly (ε-caprolactone) electrospun fibers. Biomaterials. 2006;27:2042–50.
13. Jiang H, Hu Y, Li Y, Zhao P, Zhu K, Chen W. A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents. J Controlled Release. 2005;108:237–43.
14. Rujitanaroj PO, Pimpha N, Supaphol P. Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. Polymer. 2008;49:4723–32.
15. Santoveña A, Alvarez-Lorenzo C, Concheiro A, Llabrés M, Fariña JB. Rheological properties of PLGA film-based implants: correlation with polymer degradation and SPf66 antimalaric synthetic peptide release. Biomaterials. 2004;25:925–31.
16. Fouad H, Elsarnagawy T, Almajhdi FN, Khalil KA. Preparation and in vitro thermo-mechanical characterization of electrospun PLGA nanofibers for soft and hard tissue replacement. Int J Electrochem Sci. 2013;8:2293–304.
17. Khalil KA, Fouad H, Elsarnagawy T, Almajhdi FN. Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications. Int J Electrochem Sci. 2013;8:3483–93.
18. Hong KH, Woo SH, Kang TJ. In vitro degradation and drug-release behavior of electrospun, fibrous webs of poly (lactic-co-glycolic acid). J Appl Polym Sci. 2012;124:209–14.
19. Dai W, Kawazoe N, Lin X, Dong J, Chen G. The influence of structural design of PLGA/collagen hybrid scaffolds in cartilage tissue engineering. Biomaterials. 2010;31:2141–52.
20. Kim MS, Ahn HH, Shin YN, Cho MH, Khang G, Lee HB. An in vivo study of the host tissue response to subcutaneous implantation of PLGA- and/or porcine small intestinal submucosa-based scaffolds. Biomaterials. 2007;28:5137–43.
21. Mooney DJ, Baldwin DF, Suh NP, Vacanti JP, Langer R. Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. Biomaterials. 1996;17:1417–22.
22. Abu-Saied MA, Khalil KA, Al-Deyab SS. Preparation and characterization of poly vinyl acetate nanofiber doping copper metal. Int J Electrochem Sci. 2012;7:2019–27.
23. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods. 1989;119:203–10.
24. Renner K, Amberger A, Konwalinka G, Kofler R, Gnaiger E. Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. Biochim Biophys Acta. 2003;1642:115–23.
25. Megelski S, Stephens JS, Chase DB, Rabolt JF. Micro- and nanostructured surface morphology on electrospun polymer fibers. Macromolecules. 2002;35:8456–66.
26. Fong H, Chun I, Reneker DH. Beaded nanofibers formed during electrospinning. Polymer. 1999;40:4585–92.
27. Mit-uppatham C, Nithitanakul M, Supaphol P. Ultrafme electrospun polyarnide-6 fibers: effect of solution conditions on morphology and average fiber diameter. Macromol Chem Phys. 2004;205:2327–38.
28. Jarusuwannapoom T, Hongrojjanawiwat W, Jitjaicham S, Wannatong L, Nithitanakul M, Pattamaprom C, Koombhongse P, Rangkupan R, Supaphol P. Effect of solvents on electro-spinnability of polystyrene solutions and morphological appearance of resulting electrospun polystyrene fibers. Eur Polymer J. 2005;41:409–21.
29. Dai J, Bruening ML. Catalytic nanoparticles formed by reduction of metal ions in multilayered polyelectrolyte films. Nano Lett. 2002;2:497–501.
30. Kuo PL, Chen WF. Formation of silver nanoparticles under structured amino groups in pseudo-dendritic poly(allylamine) derivatives. J Phys Chem B. 2003;107:11267–72.
31. Yu H, Xu X, Chen X, Lu T, Zhang P, Jing X. Preparation and antibacterial effects of PVA-PVP hydrogels containing silver nanoparticles. J Appl Polym Sci. 2007;103:125–33.
32. Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, Semeraro S, Turco G, Gennaro R, Paoletti S. Noncytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. Biomacromolecules. 2009;10:1429–35.
33. Henglein A. Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. J Phys Chem. 1993;97:5457–71.
34. Houchin ML, Topp EM. Chemical degradation of peptides and proteins in PLGA: a review of reactions and mechanisms. J Pharm Sci. 2008;97:2395–404.
35. Said SS, Aloufy AK, El-Halfawy OM, Boraei NA, El-Khordagui LK. Antimicrobial PLGA ultrafine fibers: Interaction with wound bacteria. Eur J Pharm Biopharm. 2011;79:108–18.
36. Zheng Z, Yin W, Zara JN, Li W, Kwak J, Mamidi R, Lee M, Siu RK, Ngo R, Wang J, Carpenter D, Zhang X, Wu B, Ting K, Soo C. The use of BMP-2 coupled-nanosilver-PLGA composite grafts to induce bone repair in grossly infected segmental defects. Biomaterials. 2010;35:9293–300.
37. Wei X, Luo M, Li W, Yang L, Liang X, Xu L, Kong P, Liu H. Synthesis of silver nanoparticles by solar irradiation of cell-free Bacillus amyloliquefaciens extracts and AgNO3. Bioresour Technol. 2012;103:273–8.
38. Liong M, France B, Bradley KA, Zink JI. Antimicrobial activity of silver nanocrystals encapsulated in mesoporous silica nanoparticles. Adv Mater. 2009;21:1684–9.
39. Yoon KY, Hoon Byeon J, Park JH, Hwang J. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. Sci Total Environ. 2007;373:572–5.
40. Maneewattanapinyo P, Banlunara W, Thammacharoen C, Ekgasit S, Kaewamatawong T. An evaluation of acute toxicity of colloidal silver nanoparticles. J Vet Med Sci. 2011;73:1417–23.
41. Liu Y, Zheng Z, Zara JN, Hsu C, Soofer DE, Lee KS, Siu RK, Miller LS, Zhang X, Carpenter D, Wang C, Ting K, Soo C. The antimicrobial and osteoinductive properties of silver nanoparticle/poly (DL-lactic-co-glycolic acid)-coated stainless steel. Biomaterials. 2012;33:8745–56.
42. Stevanović MM, Škapin SD, Bračko I, Milenković M, Petković J, Filipić M, Uskoković DP. Poly(lactide-co-glycolide)/silver nanoparticles: synthesis, characterization, antimicrobial activity, cytotoxicity assessment and ROS-inducing potential. Polymer. 2012;53:2818–28.