

Research Article

Antibacterial Activity and Cytocompatibility of PLGA/CuO Hybrid Nanofiber Scaffolds Prepared by Electrospinning

Adnan Haider,¹ Sanghwa Kwak,¹ Kailash Chandra Gupta,^{1,2} and Inn-Kyu Kang¹

¹Department of Polymer Science and Engineering, School of Applied Chemical Engineering, Kyungpook National University, Daegu 702-701, Republic of Korea

²Polymer Research Laboratory, Department of Chemistry, IIT, Roorkee 247667, India

Correspondence should be addressed to Inn-Kyu Kang; ikkang@knu.ac.kr

Received 8 December 2014; Revised 21 February 2015; Accepted 5 March 2015

Academic Editor: Tamer Uyar

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The PLGA/CuO hybrid nanofibers scaffolds were prepared via electrospinning technique. The presence of CuO in the PLGA scaffolds was confirmed by transmission electron microscope (TEM) and X-ray photoelectron spectroscopy (XPS). The scaffolds were subjected to various antibacterial and cytobiocompatibility tests. The results not only showed excellent adhesion, proliferation, and viability (live/dead staining) for fibroblastic cells but also revealed that PLGA/CuO hybrid nanofiber scaffolds inhibited both Gram-positive and Gram-negative bacterial growth. The mechanism of the antibacterial activity was concluded to be based on the CuO nanoparticles and Cu⁺⁺ ions release. It is, therefore, evaluated that PLGA/CuO hybrid nanofiber scaffolds can be a useful candidate for wound dressing.

1. Introduction

The emergence of infectious diseases due to microbes in general and the antibiotic-resistant bacterial strains (Gram-positive and Gram-negative) in particular poses a serious threat to public health worldwide [1]. Both Gram-positive and Gram-negative bacterial strains are considered a major threat to animal's life [1, 2]. In the last century, antibiotics were used as the only source for controlling and/or curing the infections caused by microbes. The current advances in the field of nanotechnology, especially, having the facility and ability to prepare metal or metal oxide nanoparticles of specific size and shape, have enabled human beings to invent new techniques for the preparation of antimicrobial agents against the microbes [2]. Microbial activity of the nanoparticles in part depends on their size, stability, and concentration of the nanoparticles in the growth medium. These properties of nanoparticles can easily be altered. Nanoparticles have therefore received great attention in the medicine field [3]. Considering the unique properties, nano-sized inorganic particles such as metal oxides of zinc (Zn), magnesium (Mg), silver (Ag), and copper (Cu) are being generated for the ultimate use in biomedical nanotechnology

[4, 5]. Antimicrobial activity of nanoparticles has largely been studied with human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* [4]. The microbial cells and their colonies showed vulnerability to metals nanoparticles such as Zn, Ag, Cu, and Mg [3, 5, 6]. The bacterial population growth is inhibited by specific interaction of these metal nanoparticles with bacterial strains [1, 6]. Among these metal nanoparticles, Cu and copper oxide (CuO) nanoparticles have attracted considerable attention because Cu is easily available and one of the most widely used metals in the modern research world [7]. Since researchers have well documented the synthesis and optical, catalytic, and electrical properties and medicinal properties of CuO nanoparticles [8, 9], this study has chosen compositing CuO with the polymer for the ease of processability and developing a hybrid antibacterial scaffold [10].

Polymeric scaffolds play a vital role in tissue regeneration [11]. They are intended to provide favorable environment to the cells that ultimately help in stimulating their growth and normal cellular functions. Polymeric scaffolds help in the movement of various growth factors, metabolites, and soluble drugs that help in stimulating cell growth and help in tissue regeneration. Poly(lactide-co-glycolide) (PLGA) has

been the most frequently used polymeric materials among the various polymeric materials used for the preparation of electrospun nanofiber scaffolds [11]. PLGA has been approved by the Food and Drug Administration (FDA) authority due to its biocompatible and biodegradable nature [12, 13]. Furthermore PLGA has been extensively studied in the field of drug delivery and tissue regeneration. Apart from the biocompatible nature, PLGA offers different degradation time by tailoring the monomer ratio of lactide and glycolide [12].

Till now various techniques have been introduced for the preparation of various polymeric scaffolds including gas forming, freeze-drying, phase separation, and solvent casting. Among the so far used techniques, electrospinning is a simple, versatile, and cost effective technique used for generating fibers with size ranging from submicron (μm) to nanometer (nm) scale diameter [13, 14]. Electrospinning is used for producing multifunctional nanofiber from both natural and artificial polymers, polymer blends, and composite [15]. Along with high surface area, inter- and intrafibrous pores, electrospun nanofiber scaffolds have a strong adhesive force, good air filtration, high adhesion barrier activity, and heat resistance [15–17]. These properties make these nanofiber scaffolds quite similar in shape to human skin. The scope of electrospun nanofiber has been explored specifically in biomedical area for various reasons such as biofilms, wound dressing materials, hemostatic materials, artificial blood vessels, drug and gene delivery, antimicrobial agent, and tissue regeneration scaffolds [3, 18].

Keeping these antecedents in mind, the purpose of this study was to test and determine the antibacterial activity of PLGA/CuO hybrid nanofiber scaffolds on various bacterial strains. Furthermore, we aimed to study the interaction of fibroblast (skin cells) with PLGA/CuO hybrid nanofiber scaffolds so that it can be used as an internal and external wound dressing agent. This kind of study will pave new ways in designing the future wound dressing materials, which can behave as an antibacterial agent without compromising its cytocompatibility.

2. Experimental

2.1. Materials and Methods. Poly(lactide-co-glycolide) (PLGA), copper (Cu), dimethylformamide (DMF), and tetrahydrofuran (THF) were purchased from Sigma Aldrich. Fibroblastic cell line (NIH3T3) was obtained from Korea Cell Bank. MTT assay kit was purchased from Gibco, Invitrogen, USA, whereas Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin G-streptomycin were acquired from Gibco, Japan. *Escherichia coli* (KCCM 12119) and *staphylococcus aureus* (KCCM 12256) were obtained from Korean Culture Center of Microorganisms (KCCM). All the reagents and chemicals in this study were used as received.

2.2. Synthesis of Copper Oxide (CuO) Nanoparticles. For the synthesis of CuO nanoparticles, 3 mg of Cu powder was added to 30 mL of distilled water in a glass vial and was

sonicated for about 25 minutes in a sonicator. The reaction mixture was transferred into a glass bottle and sealed under ordinary conditions by wrapping the bottle cap with Teflon foil. The sealed glass bottle containing reaction solution was autoclaved at 140°C for 24 hours. After 24 hours the solution was allowed to cool at room temperature and then centrifuged to retrieve CuO nanoparticles. The nanoparticles were washed repeatedly with double distilled water, freeze-dried, and stored [19].

2.3. Solution Preparation and Fabrication of Nanofiber Scaffolds. Polymer solutions at the concentration of 5 to 20 wt% were prepared by dissolving PLGA in a binary solvent of THF and DMF in 3:1 ratio. The solution was stirred overnight at room temperature until complete dissolution. The prepared solution was then subjected to electrospinning process. The typical electrospinning apparatus and the basic concept of the fiber formation are summarized in Figure 1. Briefly, the solution was transferred to a 10 mL glass syringe fitted with a needle with an inner diameter of 0.9 mm. The electrospinning experiment was based on our previously reported study [13]. The optimized electrospinning conditions used in the present study were tip to collector distance 20 cm, applied voltage 20 kV, and a solution flow rate 1 mL/h which was maintained throughout the electrospinning process. The electrospun nanofiber scaffolds were collected onto the aluminium foil wrapped over the metallic collector. After the completion of the process, the electrospun nanofiber scaffolds were removed from the metallic collector along with the aluminium foil. The electrospun nanofiber scaffold was vacuum dried overnight at 40°C to remove the solvent. The same procedure was adapted for the preparation of the electrospun PLGA/CuO hybrid nanofiber scaffolds containing 0.5 wt% of CuO nanoparticles.

2.4. Characterization of the Scaffolds. The viscosity of the PLGA polymer solutions in the binary solvent (THF : DMF = 3:1 ratio) was measured at room temperature using a viscometer (Brookfield Viscometer DV-II Pro) with spindle number 6 at 100 revolutions per minute (rpm). The morphology of the PLGA/CuO hybrid nanofiber scaffolds was evaluated by field emission scanning electron microscopy (FE-SEM, 400 Hitachi, Tokyo, Japan) and transmission electron microscope (BioTEM, Hitachi, Tokyo, Japan). The particle size was analyzed via dynamic light scattering (DLS). The qualitative and quantitative analysis of CuO, PLGA/CuO, and PLGA nanofiber scaffolds were carried out by X-ray photoelectron spectroscopy (XPS, ESCA LAB VIG microtech, Mt 500/1, etc., East Grinstead, UK) equipped with Mg K α radiation at 1,253.6 eV and a 150-W power mode at the anode. A survey scan spectrum was taken and the surface elemental compositions relative to the carbon were calculated from the peak heights taking into account the atomic sensitivity.

2.5. Antibacterial Activity. The antibacterial activities of pristine PLGA and PLGA/CuO nanofiber scaffolds were investigated against model microbial species including *Escherichia coli* (Gram-negative) and *Staphylococcus aureus*,

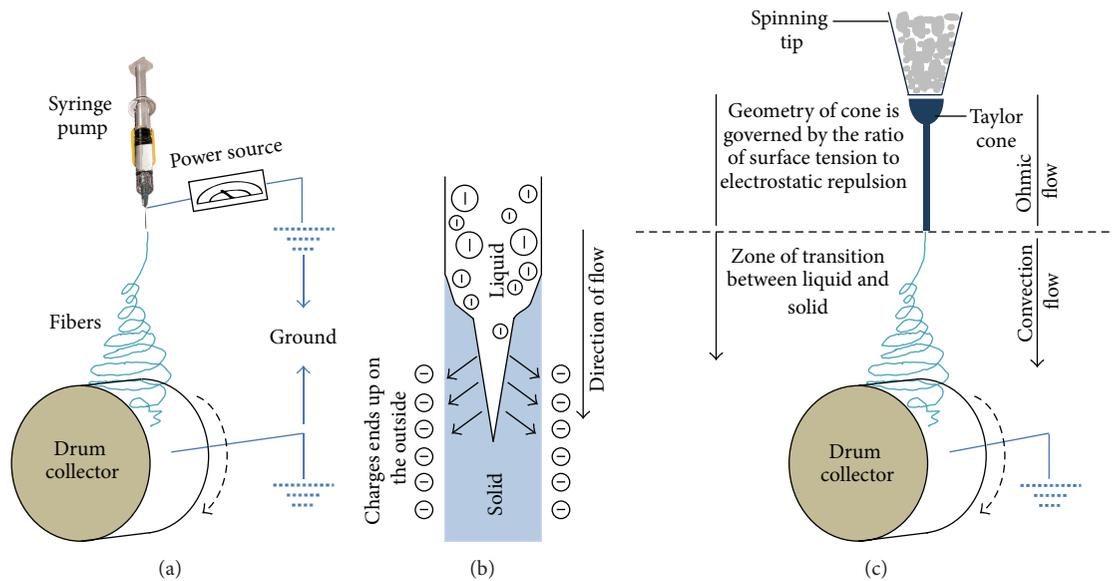


FIGURE 1: The schematic of the basic concept of electrospinning: electrospinning apparatus (a), accumulation of charges on the needle and consequently on the liquid (b), and Taylor cone and basic schematic for nanofiber preparation (c).

S. aureus (Gram-positive), through two well-known methods described below.

2.5.1. Agar Disc Diffusion Method. In this method, the antibacterial activities of the pristine PLGA and PLGA-CuO hybrid nanofiber scaffolds were measured by disc diffusion method [20]. Microbial species, *E. coli* (KCCM 12119), was grown on nutrient agar (DIFCO 0001) containing 3 g/L beef extract, 5 g/L peptones, and 15 g/L agar in distilled water, whereas *S. aureus* (KCCM 12256) was grown on trypticase soy agar (BBL 211768) containing 17 g/L pancreatic digest of casein, 3 g/L pancreatic digest of soybean meal, and 15 g/L agar in distilled water. The pH of the agar media for both microbial species was maintained at 7.0 [20]. Disc shape samples of PLGA and PLGA/CuO hybrid nanofiber scaffolds with $1 \times 1 \text{ cm}^2$ dimension were prepared and subjected to the inhibition zone test. The samples were sterilized with UV for 2 hours and subsequently placed on *E. coli* and *S. aureus* culture plates. The plates were incubated for 24 hours at 37°C . The relative antibacterial effect was found by measuring the clear zones of inhibition formed around the discs [21].

2.5.2. Optical Density Method. In this method, pristine PLGA and PLGA-CuO hybrid nanofiber scaffolds were sliced into small pieces and sterilized at 121°C for 15 minutes. Next, 50 mL of growth medium for both bacterial species was taken in 100 mL flasks, followed by addition of 0.005 g/mL finely sliced solid PLGA and PLGA-CuO hybrid nanofiber scaffolds. The tubes were then seeded with 1 mL fresh culture of bacterial strains and incubated in a shaking incubator at 37°C and for 24 hours. The turbidity of the media was observed at various time intervals at 610 nm using a UV spectrophotometer (T60U, China) [20, 22].

2.6. Cell Adhesion Study. To examine the response of the fibroblastic cells to the pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds, small circular samples of $1 \times 1 \text{ cm}^2$ dimension of both scaffolds were prepared and used. Briefly, the circular samples of the PLGA and PLGA/CuO hybrid nanofiber scaffolds were fitted in a 24-well culture dish and subsequently immersed in a DMEM medium containing 10% FBS and 1% penicillin G-streptomycin. One milliliter of a NIH3T3 cell solution (5×10^5 cells/mL) was seeded on the electrospun pristine and PLGA/CuO hybrid nanofiber scaffolds and incubated in a humidified atmosphere (5% CO_2 and at 37°C) for 1 and 3 days in order to determine the cell adhesion on the nanofiber scaffolds. After incubation, the supernatant was removed, washed twice with PBS, and fixed with an aqueous 2.5% glutardialdehyde solution for 20 minutes. The sample sheet was then dehydrated, dried in a critical point drier, and stored for characterization [3].

2.7. Cell Proliferation and Viability Study. 3-(4, 5-Dimethylazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to determine the proliferation of fibroblastic cells on the PLGA and PLGA/CuO hybrid nanofiber scaffolds. Briefly, NIH3T3 cells were seeded at a concentration of 3×10^4 cells/mL onto pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds, which were fitted in a 24-well plate, and cell proliferation was monitored after 1 and 3 days of incubation. A MTT solution (50 μL , 5 mg/mL in PBS) was added to each well and incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 4 hours. After removing the medium, the converted dye was dissolved in acidic isopropanol (0.04 N HCl-isopropanol) and kept in the dark at room temperature for 30 minutes. From each sample, 100 μL medium was taken, transferred to a 96-well plate, and

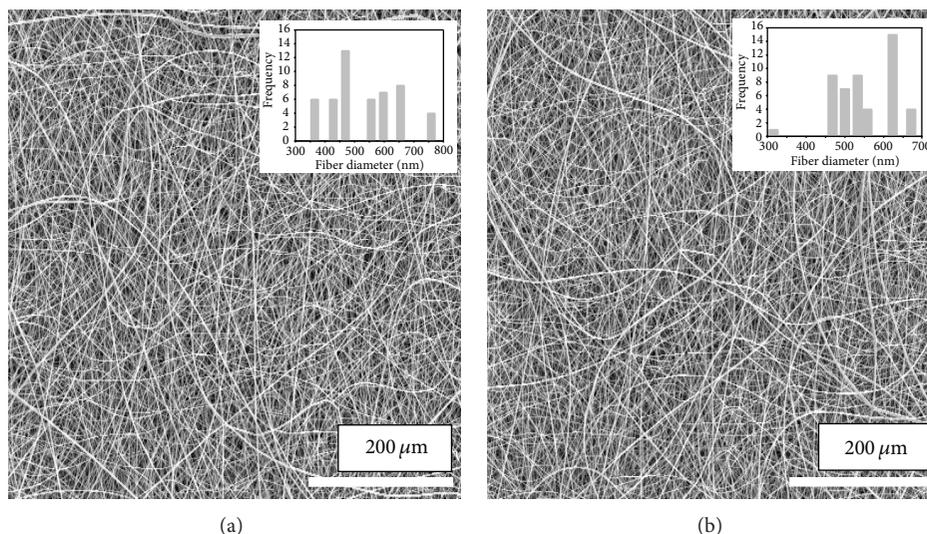


FIGURE 2: FE-SEM images of PLGA (a) and PLGA/CuO hybrid nanofiber scaffolds (b).

subjected to ultraviolet measurements for the converted dye at a wavelength of 570 nm on a kinetic microplate reader (EL \times 800, Bio-T Instruments, Inc., Highland Park, USA).

A standard live/dead assay was conducted for the evaluation of NIH3T3 cell viability after culturing NIH3T3 cells on pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds. The cell viability experiment was performed in accordance with the previous articles [3, 23].

2.8. Cu^{++} Ion Release. The Cu^{++} ions release experiment was carried out to find the ionization potential of CuO from the PLGA/CuO hybrid nanofiber scaffolds. The amount of Cu^{++} ions released from the sample was determined by immersing the PLGA/CuO hybrid nanofiber scaffolds ($4 \times 4 \text{ cm}^2$, 0.2 g) into 10 mL distilled water for different time periods. The amount of Cu^{++} ions in the double distilled water was determined by inductively coupled plasma spectrophotometer (ICP, Thermo Jarrell Ash IRIS-AP) [3].

3. Results and Discussion

3.1. Morphology of Nanofiber and CuO. Figure 2 depicts the FE-SEM images of the electrospun PLGA and PLGA/CuO hybrid nanofiber scaffolds. It is evident from the FE-SEM images of PLGA nanofiber (Figure 2(a)) and PLGA/CuO hybrid nanofiber scaffolds (Figure 2(b)) that after implementing optimized electrospinning parameters the electrospun nanofiber was smooth and uniform. The average diameters calculated from the histograms of the electrospun pristine PLGA (inset of (Figure 2(a)) and PLGA/CuO (inset of (Figure 2(b)) hybrid nanofiber scaffolds were 548 and 553 nm, respectively. This small change in the average diameter of the fibers shows that the addition of CuO to PLGA did not affect the average diameter of the fibers to a great extent.

TEM images of the CuO nanoparticles and PLGA/CuO hybrid nanofiber are depicted in Figures 3(a) and 3(b).

Images show that CuO nanoparticles (Figure 3(a)) were successfully incorporated into the PLGA nanofiber (Figure 3(b)). The size distribution of CuO nanoparticles calculated from TEM (Figure 3(a)) was ranging from 40 to 100 nm, whereas the relative size distribution obtained from DLS (inset of Figure 3(a)) was in the range of 60–128 nm with the maximum distribution peak observed at 95 nm. Hence it was concluded that the size of the CuO ranged from 40 to 128 nm.

3.2. XPS Study. ESCA survey scan spectra were used to confirm the presence of CuO nanoparticles in the matrix of PLGA electrospun nanofiber. Cu peak is the marker of choice for confirming the presence of CuO nanoparticles in the PLGA/CuO hybrid nanofiber scaffolds. The survey scan spectra of pristine PLGA nanofiber scaffolds show typical peaks of carbon C1s at 284.6 eV and oxygen O1s at 536.1 eV (Figure 4(a)). Similarly, two characteristic signals of Cu at 932.4 eV and oxygen O1s at 536.1 eV were detected in the survey scan spectra of pure CuO nanoparticles (Figure 4(c)). The appearance of the characteristic peaks of Cu, carbon, and oxygen at 932.4 eV, 284.6 eV, and 536.1 eV, respectively, in the survey scan spectra of PLGA/CuO hybrid nanofiber scaffolds confirmed the presence of CuO nanoparticles in the PLGA polymer matrix (Figure 4(b)). Besides the qualitative analysis of the survey scans from the peak positions, quantitative determination of the major elements was performed by analyzing the peak intensity and summarized in Table 1. The analysis of the data showed that after the successful incorporation of CuO nanoparticles into the PLGA/CuO hybrid nanofiber scaffold, a change in the % weights of carbon (62.10), oxygen (47.53), and CuO (0.40) for PLGA/CuO hybrid nanofiber scaffolds was observed as compared to the pristine CuO nanoparticles and PLGA nanofiber. Therefore, it was confirmed from the ESCA that CuO nanoparticles were successfully incorporated into PLGA during electrospinning to prepare PLGA/CuO hybrid nanofiber scaffold.

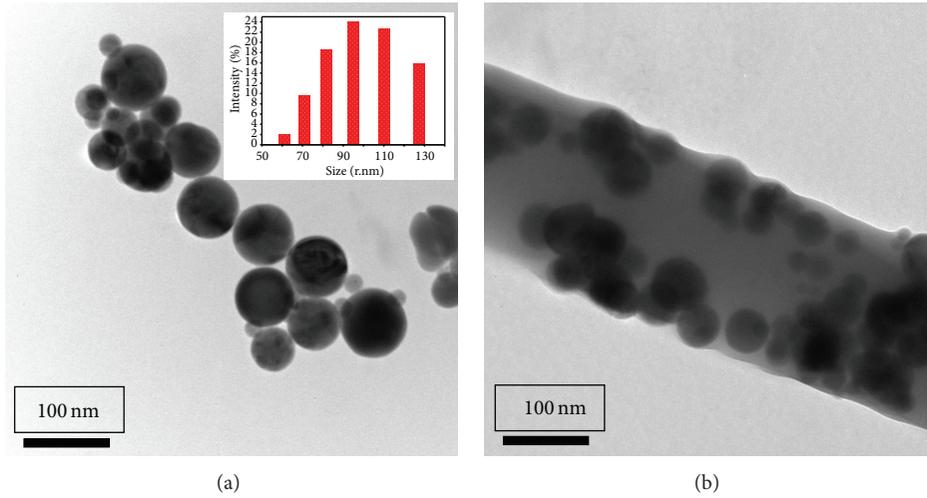


FIGURE 3: TEM images of the CuO nanoparticles (a) and PLGA/CuO electrospun hybrid nanofiber (b).

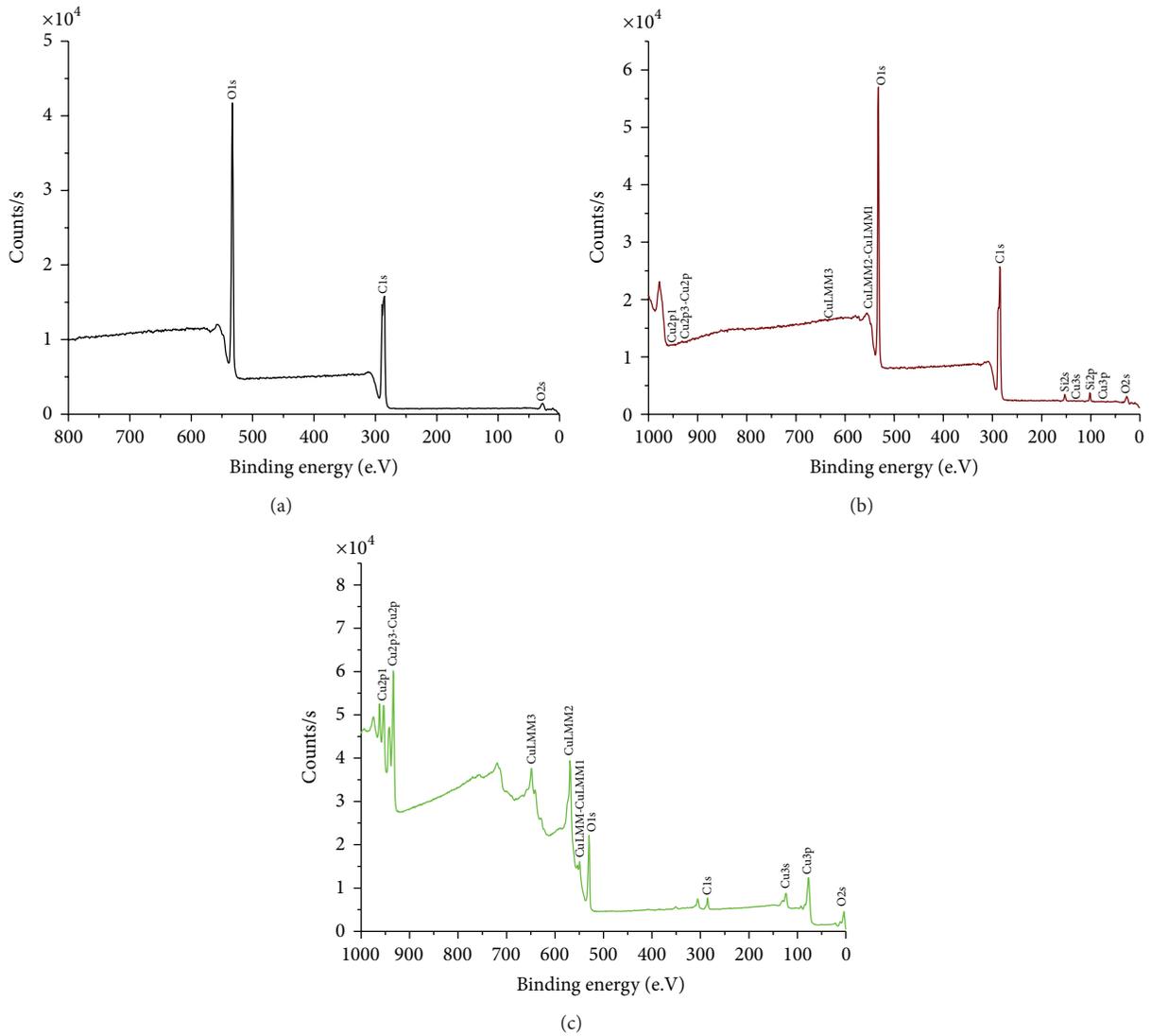


FIGURE 4: ESCA survey scan spectra of pristine PLGA (a), CuO nanoparticles (b), and (c) PLGA/CuO hybrid nanofiber scaffold.

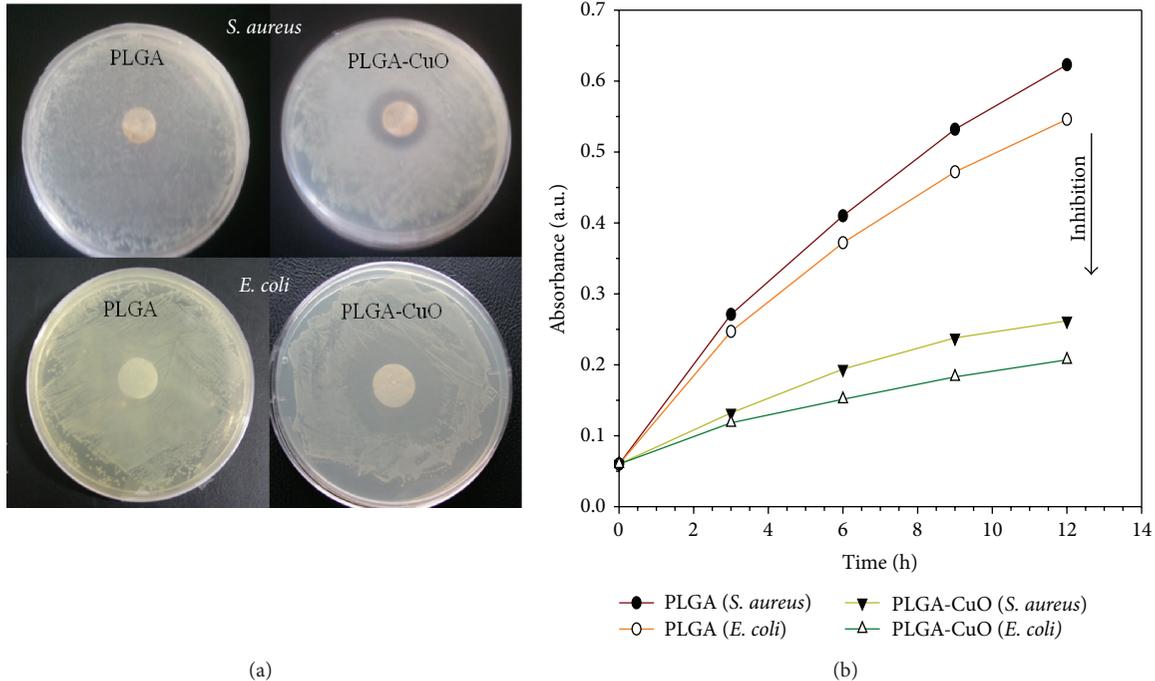


FIGURE 5: Inhibition zones of the PLGA and PLGA/CuO nanofiber scaffolds against *E. coli* and *S. aureus* determined by disc diffusion method (a) and antibacterial activities of PLGA and PLGA/CuO nanofiber scaffolds against *E. coli* and *S. aureus* determined by optical density method (b).

TABLE 1: Chemical composition of the CuO, PLGA, and PLGA/CuO composite nanofiber scaffolds calculated from ESCA survey scan spectra.

Substrates	Atomic percent (%)		
	C	O	Cu
PLGA	64.61	35.39	
CuO	6.23	43.40	49.70
PLGA/CuO	62.10	35.53	0.40

3.3. Antibacterial Properties of PLGA and PLGA-CuO Hybrid Nanofiber Scaffolds. Lack of antibacterial properties is one of the serious obstacles limiting biomedical applications of biopolymers including PLGA. As being used in wound healing and tissue regeneration, the PLGA scaffolds should keep the ability of protection against pathogenic species [12, 13]. The current PLGA/CuO hybrid nanofiber scaffolds were prepared with the aim of introducing antibacterial activities into the PLGA electrospun nanofiber so as to enhance their applicability in medical field. Figures 5(a) and 5(b) illustrate the comparative results of the antibacterial activities of pristine PLGA and PLGA/CuO nanofiber scaffolds. The results obtained from disc diffusion method against *E. coli* and *S. aureus* indicated that the pristine PLGA nanofiber scaffold did not produce any zone of inhibition against both species (*E. coli* and *S. aureus* (Figure 5(a)). Thus the results clearly demonstrate that the pristine PLGA has nonbactericidal nature. On the other hand, the PLGA/CuO hybrid nanofiber scaffold produced zones of inhibition against both *E. coli*

and *S. aureus* (Figure 5(a)) indicating antibacterial activity based on the incorporation of CuO into PLGA nanofiber. Thus PLGA/CuO hybrid nanofiber scaffold has obtained the bactericidal ability. A similar phenomenon was also indicated by the optical density method (Figure 5(b)). An increasing trend in the cell density is observed in the samples containing PLGA; however, the growth of both the *E. coli* and *S. aureus* is greatly inhibited by PLGA/CuO nanofiber scaffold. The inhibition trend remained the same as was shown by disc diffusion method; that is, *E. coli* is inhibited more as compared to *S. aureus*. Thus it is concluded that the disc diffusion as well as optical density method showed significant (Figures 5(a) and 5(b)) antibacterial activity of PLGA/CuO nanofiber scaffold. The antibacterial activities of Cu and CuO are well established [24]. CuO, not only in colloidal form but also in composite form, have also shown tremendous antibacterial properties [20, 25]. Herein the produced inhibition zones and the higher inhibition in the growth of both *E. coli* and *S. aureus* could be attributed to the bactericidal nature of CuO [26–28].

Schematics of the potential mechanisms which can be effective in retarding the bacterial growth are depicted in Figure 6. Exact mechanism of the antibacterial activities of nanomaterials is still not known [2, 29]. The bacterial growth can be inhibited by the penetration of CuO nanoparticles or Cu^{++} ions into the cell via cell membrane or may inhibit bacterial growth by the reactive oxygen species (ROS) generation. However, the most established mechanism is the adhesion of CuO nanoparticles/ Cu^{++} ions to the protein containing sulphur present in the cell wall of the bacterial

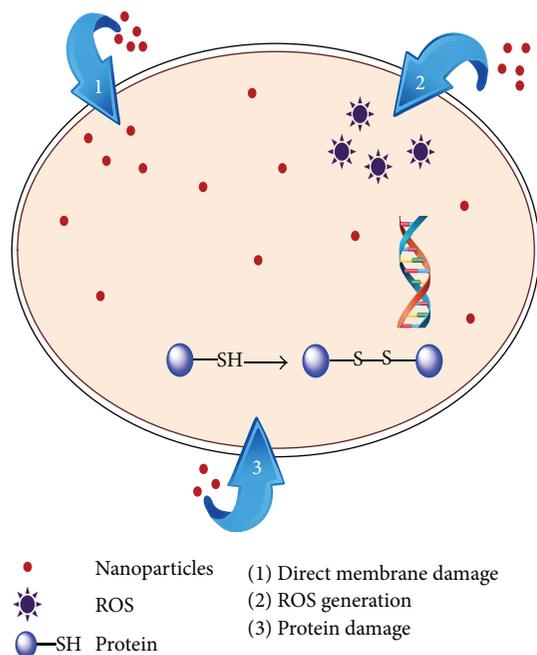


FIGURE 6: Schematic representing mechanism related to the antibacterial activity of CuO nanoparticles/Cu⁺⁺ ions.

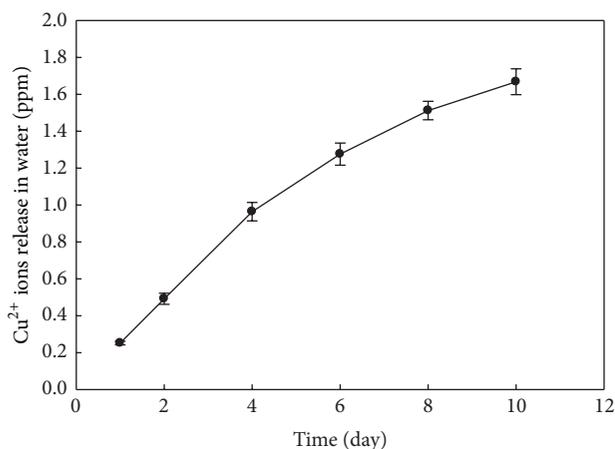


FIGURE 7: Cu⁺⁺ ions release in water from the PLGA/CuO hybrid nanofiber scaffolds with respect to incubation time.

cells, which leads to the malfunctioning of the bacterial cell wall and ultimately causes the bacterial cell death [27, 28, 30].

3.4. Cu⁺⁺ Ions Release Study. Figure 7 depicts the results obtained from the Cu⁺⁺ ions release experiment. It was observed that Cu⁺⁺ ions from the PLGA/CuO hybrid nanofiber scaffold were released in a sustained manner, therefore enabling the PLGA/CuO hybrid nanofiber scaffolds to exhibit antibacterial effect for longer duration. The amount of Cu⁺⁺ ions released from the PLGA/CuO hybrid nanofiber scaffold reached 1.6 ppm after 10 days of incubation in water. The Cu⁺⁺ ions release and the inhibition of *E. coli* and *S. aureus* by the PLGA/CuO nanofiber scaffold (Figure 5(b))

follow the same pattern; this is an indication that the operating mechanism of inhibition is governed by Cu⁺⁺ ions. However, for Cu⁺⁺ ions release, the choice of media is important, as the body fluid has a complex composition and the components comprising body fluid exhibit different binding abilities to Cu⁺⁺ ions [28, 31, 32]. The amount of Cu⁺⁺ ions in the double distilled water was determined by inductively coupled plasma spectrophotometer.

3.5. Cytocompatibility Study

3.5.1. Cell Adhesion. NIH3T3 cells tend to get adhered to the surface of the biocompatible materials; therefore the various cell fate processes including proliferation, migration, apoptosis, and differentiation are highly affected by cells adhesion to cell-binding epitopes in the extracellular matrix (ECM) [33]. Figure 8 depicts the FE-SEM images of the NIH3T3 cells adhered to the pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds with different incubation time. It is clearly visible that NIH3T3 cells were adhered to both pristine PLGA (Figures 8(a) and 8(c)) and PLGA/CuO hybrid nanofiber scaffolds (Figures 8(b) and 8(d)), thus proving that both pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds provided good cytocompatibility environment for NIH3T3 cells. The spreading pattern of the NIH3T3 cells on the PLGA and PLGA/CuO hybrid nanofiber scaffolds further confirmed the noncytotoxic nature of the scaffolds. Furthermore, the observed increase in the number of adhered cells to the scaffolds was directly proportional to the increase in incubation time (Figures 8(c) and 8(d)) [3, 12].

3.5.2. Cell Proliferation and Viability. Many colorimetric methods have been adapted in order to estimate the exact cell number, among which the methods based on cells metabolic viability test are widely employed [34]. MTT reagents are widely used for this purpose. Figure 9 illustrates the data obtained from MTT assay of the proliferation of fibroblastic cells seeded on the PLGA and PLGA/CuO hybrid nanofiber scaffolds. From the MTT assay it was observed that NIH3T3 cells proliferated on the PLGA and PLGA/CuO hybrid nanofiber scaffolds. The NIH3T3 cells proliferation on the PLGA/CuO hybrid nanofiber scaffolds was in the range of standard deviation compared with pristine PLGA nanofiber; thus both scaffolds exhibit cytocompatibility nature. Furthermore, MTT assay was conclusive in elucidating the proliferation of NIH3T3 cells on the pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds.

Figures 10(a) and 10(b) show the fluorescence images of the NIH3T3 cells cultured on the PLGA/CuO hybrid nanofiber scaffold for 8 days using the live/dead assay [23]. The fluorescence color of cells cultured on the PLGA and PLGA/CuO hybrid nanofiber scaffolds was totally green, indicating a good viability of the fibroblastic cells. Rarely, few dead cells showed red fluorescence of propidium iodide staining (Figure 10(b)) when cultured on the PLGA/CuO hybrid nanofiber scaffolds [3]. These observations of the live/dead assay clearly suggest that the PLGA/CuO nanofiber

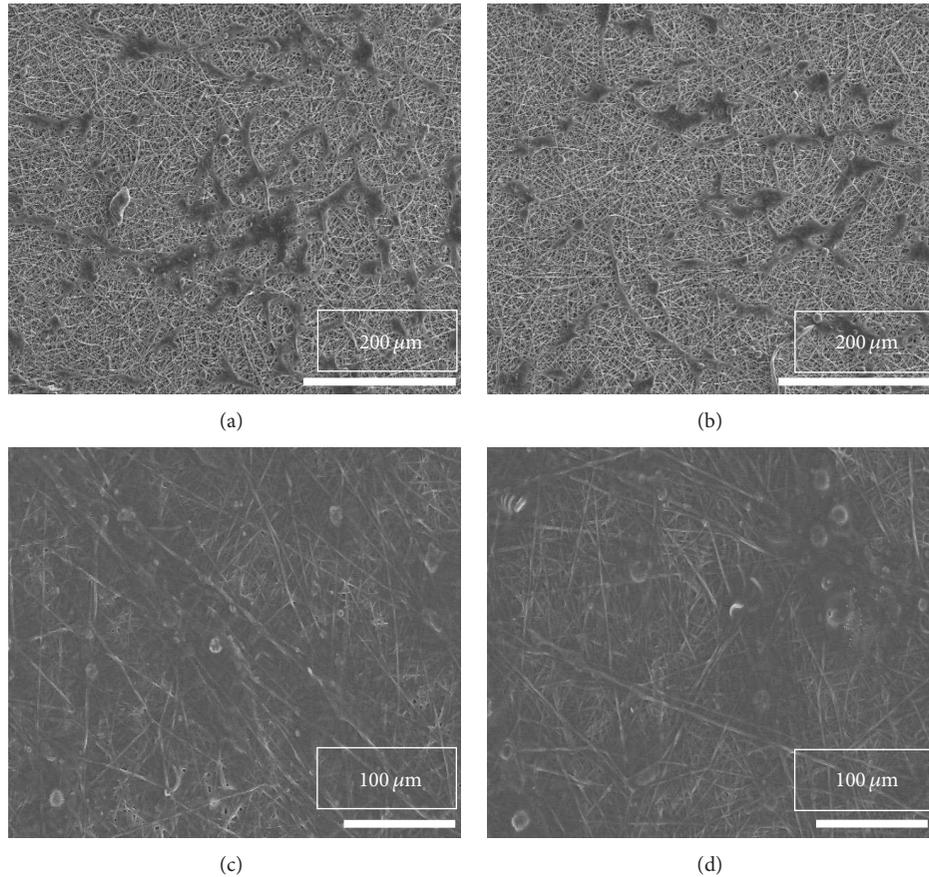


FIGURE 8: FE-SEM images of NIH3T3 cells adhered to the pristine PLGA (a, c) and PLGA/CuO hybrid nanofiber scaffolds (b, d) after 1 and 3 days of incubation.

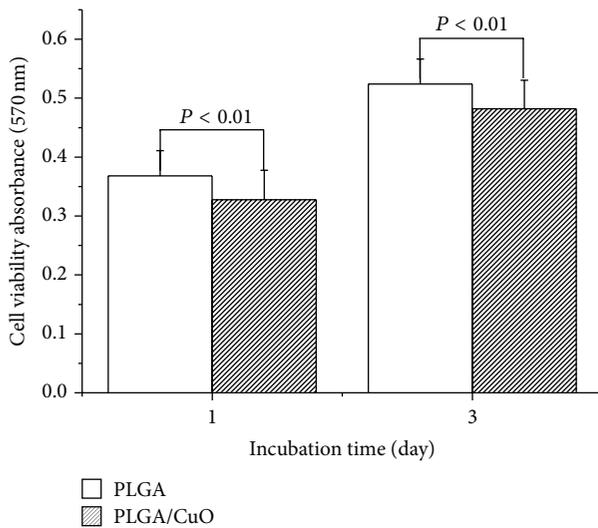


FIGURE 9: MTT assay after culturing of fibroblastic cells on the pristine PLGA and PLGA/CuO after 1 and 3 days of incubation.

scaffolds provided favorable environment to NIH3T3 cells [3].

4. Conclusion

This study was designed to evaluate the antibacterial activity along with assessing the biocompatibility of the pristine PLGA and PLGA/CuO hybrid nanofiber scaffolds. Prior to the evaluation of the antimicrobial activity, PLGA and PLGA/CuO hybrid nanofiber scaffolds were characterized using FE-SEM, TEM, and XPS. PLGA and PLGA/CuO nanofiber scaffolds showed excellent antibacterial activity against *E. coli* and *S. aureus* bacterial strains. The mechanism of the antibacterial activity was concluded to be based on the Cu^{++} ion release. Furthermore, the various tests performed for the assessment of cytocompatibility using NIH3T3 cells showed good cytocompatibility for both PLGA and PLGA/CuO nanofiber scaffolds. It is therefore concluded from the antibacterial tests and in vitro cell experiments that PLGA/CuO nanofiber scaffolds have the potential to be used as a wound dressing material.

Conflict of Interests

The authors have no conflict of interests regarding the publication of this paper.

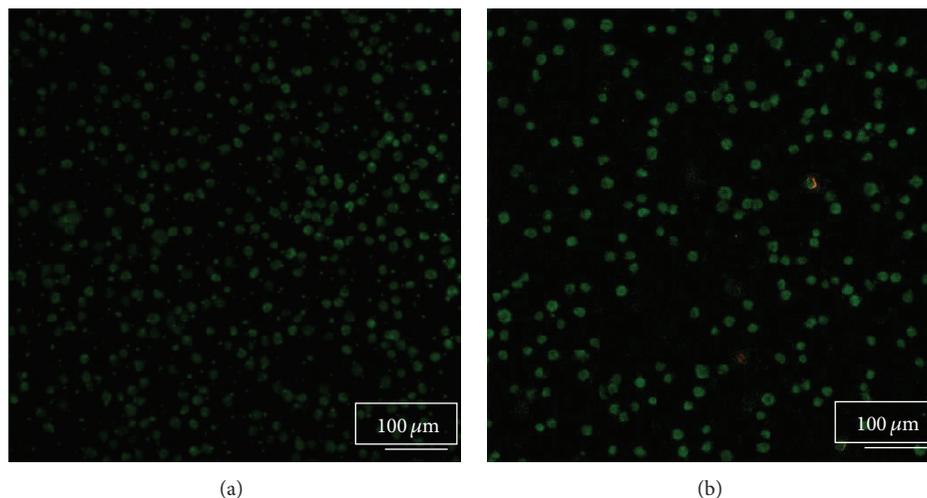


FIGURE 10: Fluorescence images of NIH3T3 cells cultured on pristine PLGA (a) and PLGA/CuO (b) fibroblastic cells after 3 days of culture.

Acknowledgments

This work was supported by the General Research Program (2013 R1A1A 2005148) and the Basic Research Laboratory program (no. 2011-0020264) from the Ministry of Education, Science and Technology of Korea.

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