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Research Article

In Vitro Assessment of Antibacterial Activity and Cytocompatibility of Quercetin-Containing PLGA Nanofibrous Scaffolds for Tissue Engineering

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Flavonoids, such as quercetin, have been reported to exhibit a wide range of biological activities related to their antioxidant capacity. The aim of this study was to investigate the protective effect of quercetin on cell adhesion, and the viability and proliferation of KB epithelial cells. Quercetin- (1, 5 wt%)-containing poly (l-lactide-co-glycolide) (PLGA) nanofibrous scaffolds (PLGA/Q 1, PLGA/Q 5) were prepared by electrospinning technique and their antibacterial properties were examined. Two types of bacteria strains, *Staphylococcus aureus* (SA) and *Klebsiella pneumoniae* (KP), were used to evaluate the antibacterial properties of the scaffolds. The results showed that the quercetin-containing PLGA nanofibrous scaffolds exhibited significant antibacterial effects against the two bacterial strains. KB epithelial cells were also used to evaluate the cytocompatibility of the scaffolds. From the results, it was found that the PLGA nanofibrous scaffolds with 1 wt% of quercetin had good cell compatibility. It is considered that the PLGA nanofibrous scaffolds with 1 wt% quercetin have potential to be used in tissue engineering.

1. Introduction

Dietary antioxidants, including polyphenolic compounds, are considered beneficial because of their potential protective role in the pathogenesis of multiple diseases associated to oxidative stress such as cancer, coronary heart disease, and atherosclerosis [1]. Flavonoids comprise a large group of naturally occurring low molecular weight polyphenolic compounds that are present in all plants [2]. Flavonoids, especially flavonols such as quercetin, have been reported to exhibit a wide range of biological activities [3], including anticarcinogenic, anti-inflammatory, and antiviral actions.

The flavonoid used in this study, quercetin, is one of the most abundant flavonol-type flavonoids present in several components of a human's diet such as vegetables, fruit, tea, and wine [3] with a well-characterized antioxidant activity *in vitro* [4, 5]. Quercetin exhibits a wide range of physiological and pharmacological activities relevant to human health, such as anticarcinogenic, anti-inflammatory, and antiviral actions [6, 7]. Many of these effects are supposed to be related to its antioxidant property. The mode of action for quercetin is scavenging free radicals through chelating divalent cations which inhibits some enzymes and protect the DNA damage. Therefore, quercetin may be considered as an effective attenuating factor for preventing various disorders caused by environmental contaminants [8–10].

Electrospun nanofiber matrices have been widely used for tissue regeneration of blood vessels [11, 12], skin [13, 14], cartilage [15, 16], bone [17, 18], and nerve [19]. Poly (l-lactide-co-glycolide) (PLGA) has been approved for several biomedical applications in humans and widely used as a scaffold material in tissue engineering [20–23]. PLGA nanofibers have been shown to promote the adhesion of interstitial and endothelial cells [24], the growth of fetal pulmonary cells

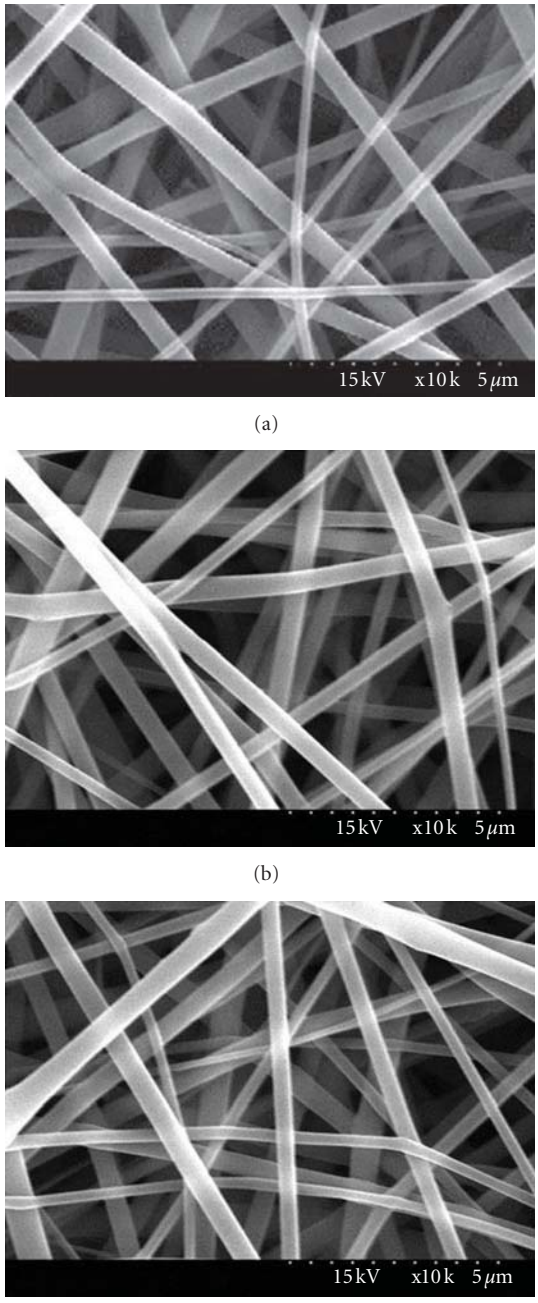


Figure 1: SEM micrographs of electrospun (a) PLGA, (b) PLGA/Q 1, and (c) PLGA/Q 5 nanofibrous scaffolds.

[25] and porcine chondrocytes [26]. Our previous study [27] showed that the silver-containing PLGA nanofibrous scaffolds inhibited the proliferation of *Staphylococcus aureus* (SA) and *Klebsiella pneumoniae* (KP) bacteria, without exhibiting any *in vitro* cell cytotoxicity. However, to date, no report has yet been published showing the antibacterial activity and cytotoxicity of quercetin-containing PLGA nanofibrous scaffolds using the electrospinning technique.

In this study, the antibacterial and biological properties of the quercetin-containing PLGA nanofibrous scaffolds

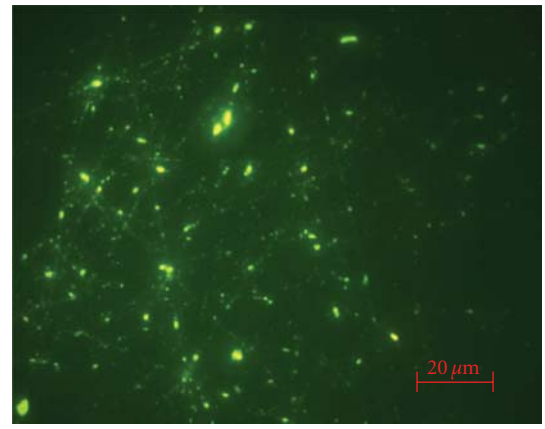


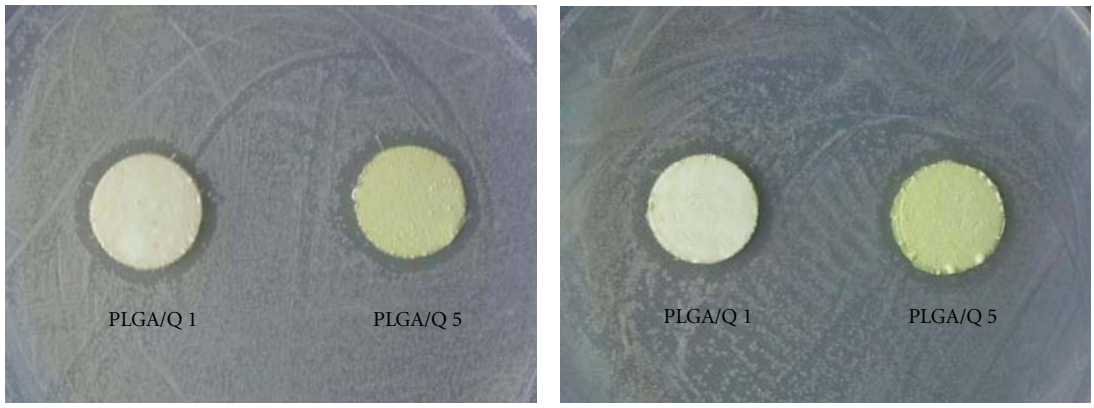
Figure 2: The fluorescence image of PLGA/Q 5 nanofibrous scaffolds.

were studied. Two types of bacterial strains, *Staphylococcus aureus* (SA) and *Klebsiella pneumoniae* (KP), were chosen to evaluate the antibacterial activity of the quercetin-containing PLGA nanofibrous scaffolds. Furthermore, *in vitro* cytotoxicity was examined by conducting tests on the scaffolds as well.

2. Materials and Methods

2.1. Preparation of Polymer Solution. Poly (l-lactide-co-glycolide) (PLGA, polymer composition: 79 : 21 ~ 85 : 15 molar ratio, Aldrich Chemical Co., USA) was dissolved in 1,1,1, 3,3,3,-hexafluoro-2-propanol (HFIP) with a concentration of 4 wt % and the solution was stirred overnight at room temperature to ensure complete dissolution. Then, certain amounts of quercetin dihydrate (Q, Sigma-Aldrich, USA) (1, 5 wt %) were mixed with the PLGA solution and stirred by magnetic stirrer for 24 h to obtain the PLGA/Q solution. The quercetin-containing PLGA nanofibrous scaffolds were named PLGA/Q 1 and PLGA/Q 5, respectively.

2.2. Electrospinning. The electrospinning experiments were done at room temperature and the apparatus for the electrospinning was assembled based on a study done by Lee et al. [28]. The polymer solution was placed into a 10 mL glass syringe fitted with a needle (20 G). A clamp connected to a high voltage power supply that could supply a positive voltage from 0 to 50 kV was attached to the needle. A piece of aluminum foil was placed at a distance of 15 cm away from the needle tip. The polymer jets generated from the needle by high voltage flew to the collector and formed a nanofiber mesh. The polymer solutions were electrospun with a fixed mass flow rate of 1.0 mL/h and a voltage of 10 kV. Finally, the electrospun samples were dried overnight at 40°C to remove the solvent. The morphologies of the electrospun nanofibers were observed by field emission scanning electron microscope (FE-SEM S-4300, Hitachi, Japan) and the fluorescence images of the electrospun nanofibers (PLGA/Q 5) were obtained using a fluorescence microscope (Carl zeiss, Germany).



(a) (b)

Figure 3: Effect of the quercetin on the formation of inhibition zones: (a) *Staphylococcus aures* and (b) *Klebsiella pneumonia*.

2.3. Antibacterial Assessment. For the zone of inhibition screening test for antibacterial activity, *Staphylococcus aureus* (SA 6538) and *Klebsiella pneumoniae* (KP 4352) were cultivated in a nutrient broth for 24 h in a CO_2 incubator. Afterwards, diluted bacteria suspensions collected from each vial were spread onto nutrient broth agar plate. After that, the PLGA/Q 1 and PLGA/Q 5 nanofibrous mats were each placed onto a lawn *Staphylococcus aureus* and *Klebsiella pneumoniae* on the agar plates and incubated overnight at 37°C. The zone of inhibition formed around the nanofibrous mats was observed against the two microorganisms.

2.4. Quercetin Release. The quercetin release test was done by immersing the PLGA/Q 5 nanofibrous scaffold ($4 \times 4\,cm^2$, 0.02 g) into distilled water (5 mL, pH 7.2) for different periods of time. The amount of quercetin released from the sample was measured by UV-visible spectra (JASCO V-650).

2.5. Cell Attachment. In order to examine the interaction of the nanofibrous scaffolds with KB epithelial cells, circular nanofibrous scaffolds were fitted in a 24-well culture dish and subsequently immersed in a DMEM medium containing 10% fetal bovine serum (FBS) (Gibco, Japan) and 1% penicillin G-streptomycin (Gibco, Japan). To the scaffolds, 1 mL of the cell solution (3×10^4 cells/cm^2) was added and incubated in a humidified atmosphere of 5% CO_2 at 37°C for 4 h. After incubation, the supernatant was removed, washed twice with PBS, and fixed in a 2.5% glutaraldehyde aqueous solution for 20 min. The sample sheet was then dehydrated, dried in a critical point drier, and finally sputter-coated with gold. The surface morphology of the samples was then observed with FE-SEM.

2.6. Cell Proliferation. The proliferation of KB epithelial cells was determined using a colorimetric immunoassay, enzyme-linked immunosorbent assay (ELISA). The ELISA method was based on the measurement of 5-bromo-2-deoxyuridine (BrdU), which was incorporated during DNA synthesis. The ELISA was done according to the manufacturer's instructions (Roche Molecular Biochemicals).

2.7. Cell Viability. A standard live/dead assay [29] was used to image cell survival, adhesion, and spatial organization. KB epithelial cells were collected by centrifugation and then were incubated in calcein-AM (1 mM in DMSO) and propidium iodide (PI, 1.5 mM in H_2O) solution for 15 min. Cells with compromised membranes exhibited red-fluorescence from the intercalation of the propidium iodide into the DNA double helix. Cells with intact cell membranes were able to use nonspecific cytosolic esterases to convert nonfluorescent calcein-AM into bright green-fluorescent calcein. Cells were observed under a fluorescence microscope using a band-pass filter (Nikon Eclipse E600-POL, Japan).

3. Results and Discussion

3.1. Surface and Morphology of Nanofibers. Figure 1 shows SEM images of the electrospun nanofibers from 4 wt% PLGA solutions with different amounts of quercetin (0, 1, and 5 wt %). It was observed that the diameter distribution of the PLGA nanofibers did not change when the quercetin was added to the PLGA solution (Figure 1). Figure 2 shows the fluorescence images of quercetin which randomly dispersed through the PLGA nanofibrous scaffold.

3.2. Antibacterial Activity. For the zone of inhibition screening test for the antibacterial activity, PLGA/Q 1 and PLGA/Q 5 nanofibrous scaffolds were placed onto a lawn of organisms on an agar plate and incubated for 24 h. After incubation, antibacterial responses were observed in both test cases. The bactericidal activity displayed a good zone of inhibition, that is, the region in which the bacterial species were unable to propagate around the PLGA/Q fiber mats (Figure 3). In addition, the growth inhibition rate of the bacteria increased a little with an increase in quercetin content (Figure 3). This is attributed to trace amounts of quercetin released from the fiber, which attached to the bacteria and inhibited nucleic acid synthesis, cytoplasmic membrane function and energy metabolism [30].

The antibacterial activity of the scaffolds is based on the role of the quercetin that was embedded in the surface of the scaffold or released from the scaffold. Therefore, in this study,

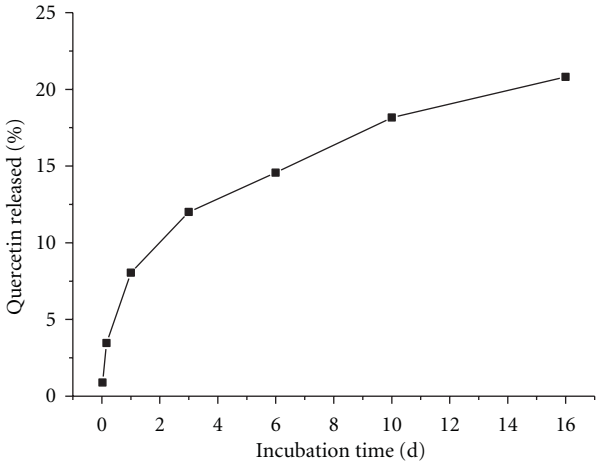


Figure 4: The percentage of quercetin released from PLGA/Q 5 nanofibrous scaffolds as a function of the immersion time.

the amount of quercetin released from the PLGA/Q 5 nanofibrous scaffold was calculated using UV-visible spectra. As shown in Figure 4, the percentage of released quercetin gradually increased with an increase in incubation time, and it reached 20.8% after 16 days of incubation. Rapid release of quercetin from PLGA/Q 5 is probably due to the high surface area of the nanofibrous scaffold. The released quercetin from the nanofibrous scaffold showed a high inhibitory effect on the growth of bacteria and killed the microorganisms, as seen in Figure 3.

3.3. In Vitro Cell Compatibility. As seen in Figure 5, KB epithelial cells were attached on the surface of three different scaffolds, and the number of cells attached on the surface of the PLGA nanofibrous scaffolds (Figure 5(a)) was not much different from those the quercetin-containing PLGA nanofibers (Figures 5(b) and 5(c)). The cells formed monolayers on the surface of the PLGA and PLGA/Q 1 nanofibrous scaffolds (Figures 6(a) and 6(b)) after 3 days of incubation. However, the monolayers were partially observed when the cells were cultured on the surface of the PLGA/Q 5 nanofibrous scaffolds (Figure 6(c)). Comparable results for differences in proliferation behavior, expressed as the amount of newly synthesized DNA, are shown in Figure 7. The cell proliferation on the quercetin-containing PLGA nanofibrous scaffolds (PLGA/Q 5) was significantly lower ($P < 0.05$) than that on the PLGA nanofibrous scaffolds (Figure 7) due to the antioxidant property of quercetin. The viability of cells cultured for 7 days on the nanofibrous scaffolds was observed by using the live/dead assay and the results are shown in Figure 8. The fluorescence colors of cells cultured on the PLGA and PLGA/Q 1 were green, indicating good viability of the cells. However, a few dead cells were found in the case of the PLGA/Q 5 nanofibrous scaffolds, which emitted a red fluorescence.

Quercetin has been reported to be a potent inhibitor of lipoxygenase (LOX) and cyclooxygenase (COX) activities, sodium-potassium adenosine triphosphatase (Na-K-ATPase), protein kinase C (PKC), and various tyrosine kinases [31]. Through one or more of the above biochemical

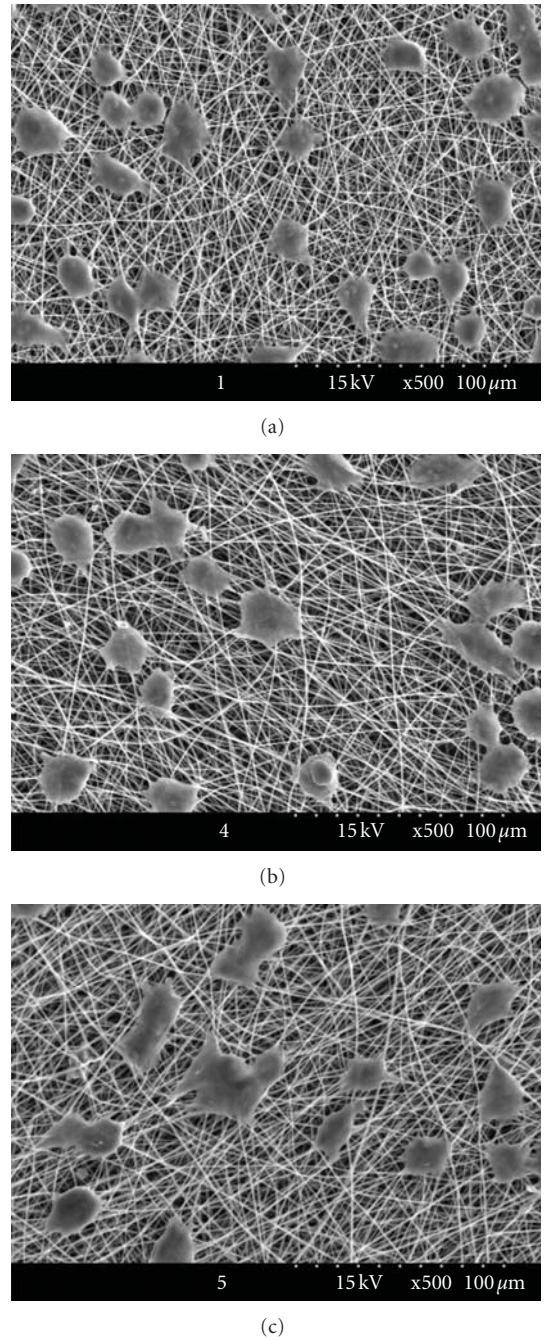


Figure 5: SEM images of KB epithelial cells cultured for 4 h on (a) PLGA, (b) PLGA/Q 1, and (c) PLGA/Q 5 nanofibrous scaffolds.

mechanisms, quercetin has been reported to protect against both chemically induced and spontaneously formed tumors in animals [31, 32] and arrest cell proliferation in a variety of transformed cell lines in culture [32–36]. Alía et al. [5] concluded that doses of quercetin significantly decreased HepG2 cell proliferation, which has been previously reported for other cancer cells *in vitro*, such as human adenocarcinoma [37], human promyelocytic leukemia [38, 39], human colon cancer [33, 34, 40], and murine hepatoma [41]. Still other investigators have found both preoxidant and antioxidant

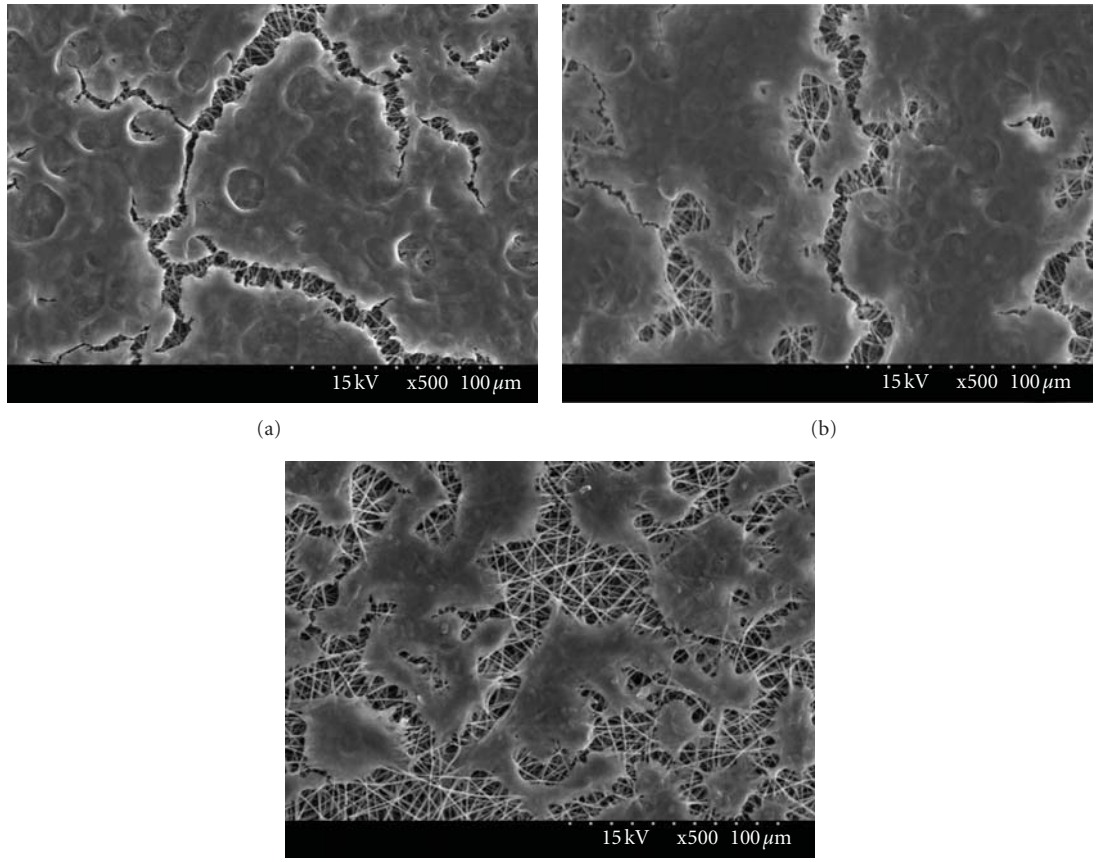


Figure 6: Morphology of KB epithelial cells incubated for 3 days on (a) PLGA, (b) PLGA/Q 1, and (c) PLGA/Q 5 nanofibrous scaffolds.

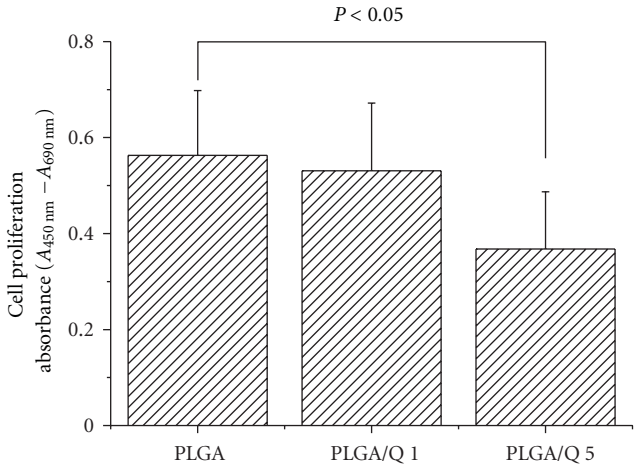
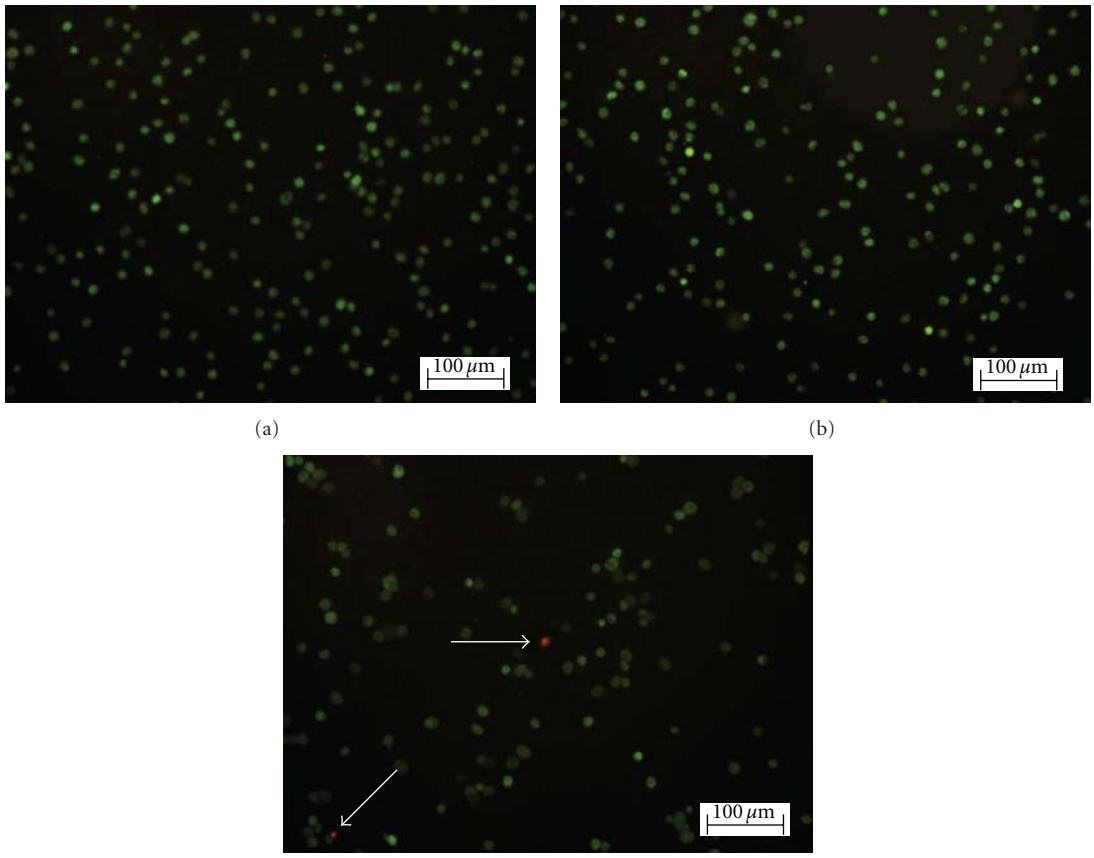


Figure 7: Proliferation of KB epithelial cells cultured for 3 days. Data are expressed as the means ± SD ($n = 6$) for the specific absorbance.

effects of quercetin depending on the concentrations used for experimentation [42]. In this study, the results showed that the quercetin-containing PLGA nanofibrous scaffolds affected the *in vitro* growth of the KB epithelial cells. Interestingly, the inhibitory effect of PLGA/Q 1 on the growth of KB epithelial cells was significantly low, indicating a lower activity or accessibility to the cultured cells. However, PLGA/Q 5 significantly reduced the proliferation of KB epithelial cells (Figure 7) due to the cytotoxic effect of the quercetin.

4. Conclusions

Quercetin-containing PLGA nanofibers were successfully prepared using an electrospinning technique and were characterized by SEM and fluorescence microscope. The results demonstrated that the nanofibers electrospun at maximum conditions were straight and that quercetin was distributed throughout the fibers. Finally, the PLGA nanofibrous scaffolds with 1 wt % of quercetin (PLGA/Q 1) inhibited the proliferation of *Staphylococcus aureus* (SA) and *Klebsiella pneumoniae* (KP) bacteria, without any *in vitro* cell cytotoxicity from the scaffolds. If these results can be confirmed *in vivo*, the PLGA/Q 1 nanofibrous scaffolds may be applicable for use in total joint arthroplasty particularly due to its effect against multiresistant bacteria.



(a) (b) (c)

Figure 8: Fluorescence microscopic images of KB epithelial cells cultured for 7 days on (a) PLGA, (b) PLGA/Q 1, and (c) PLGA/Q 5 nanofibrous scaffolds.

Acknowledgments

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