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Research Article

Preparation of Polyamide 6/CeO_2 Composite Nanofibers through Electrospinning for Biomedical Applications

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Polyamide 6 (PA6)/CeO_2 composite nanofibers were prepared by electrospinning technique. The morphological, structural, and mechanical properties of the PA6/CeO_2 nanofibers were investigated by using SEM, XRD, Fourier transform-infrared (FT-IR) spectroscopy, and an electronic universal tensile testing machine. SEM images revealed that the nanofibers were well oriented and had good incorporation with CeO_2, the average diameter of composite fibers first decreases and then increases with the loading of CeO_2. The crystallinity of fibers decreases after the addition of CeO_2. The tensile strength of the fibers first increases and then decreases with the increasing concentration of CeO_2. The proliferation properties of mouse macrophages and osteoblasts on the PA6/CeO_2 nanofibers were analyzed by an in vitro cell compatibility test, the results show that PA6/CeO_2 composite fibers is nontoxic to macrophages and osteoblasts and has good biocompatibility.

1. Introduction

As a biodegradable, biocompatible, and synthetic polymeric kind of material, polyamide 6 (PA6) has good mechanical and physical properties, is nontoxic, and has wear resistance [1, 2]. Due its chemical structure, and since the reactive group is similar to collagen, PA6 is also widely used as a biological material, such as surgical sutures, artificial blood vessels, artificial muscles, and artificial bone. However, as for biomedical materials, although PA6's comprehensive properties have been relatively perfect, it needs further surface modification to increase the multifunctional applications [3, 4].

Electrospinning is an efficient technique to fabricate continuous fibers with high surface area-to-volume ratio and high porosity [5, 6]. The high application potential of electrospinning to biological polymers has increased since the electrospun membranes were regarded as a candidate for tissue engineering constructs [7, 8]. PA6 and its composites could also be prepared or has increased multifunctional applications as nanoscale materials by electrostatic spinning under suitable conditions, when dissolved in highly polar solvent, such as formic acid [9, 10]. Abdalhay et al. [11] found that the average diameters of HAp/PA6 fibers firstly decreased and then increased with the increase of HAp content. Ahn et al. [4] observed that the concentration of PA6 solution had an important effect on the nanostructure of ES products. Pant et al. [12] observed that standing time could influence the diameter of Ag/PA6 nanofibers in the pretreatment of blend solution of $AgNO_3$/PA6. Nirmala et al. [13, 14] investigated PA6/chitosan composite nanofibers that were successfully prepared via electrospinning with diameters of about 20 to 40 nm. In a word, it can be seen that from the above analyses, the functional properties of PA nanofibers can be improved by adding crosslinking agents like HAp, Ag, and chitosan. However, since the biological activity of PA6 is insufficient, new bioactive nanoparticles need to be further explored to increase the biological activity and other multifunctional applications of PA nanofibers.

Cerium oxide (CeO_2), a rare earth metal oxide of the lanthanide series, has a face centered cubic fluorite-type crystal

structure and exists in two oxidation states +3 and +4 [15, 16]. The Ce^{3+}/Ce^{4+} redox switch and oxygen vacancies by surface defects make the CeO_2 nanoparticles have redox and biological activities, and can promote the growth and differentiation of osteoblasts, and improve the biological activity and antioxidant and anti-inflammatory ability of the biological materials [17–19]. Gojova et al. [20] when studying the effect of metal particles on inflammatory response of vascular endothelial cells found that cerium dioxide has a certain anti-inflammatory ability. Hirst [21] found that cerium dioxide has good biocompatibility and can inhibit inflammatory response by scavenging free radicals and reactive oxygen species. However, with the nanoization of CeO_2, it also has its own defects. On one hand, the smaller is the particle size of CeO_2, the more atoms on the surface, leading to the higher surface energy and the easier to form aggregates. On the other hand, as a kind of a biological material, the biological safety of CeO_2 nanomaterials has also gradually attracted our attention.

In this paper, we considered combining PA6 and CeO_2 with electrospinning in order to obtain biomedical nanofibers, which would be used as biological materials, such as surgical sutures, artificial blood vessels, artificial muscles, and artificial bone. Recent papers have already reported on the modification of the functional properties of PA nanofibers; here, CeO_2 nanomaterials are used to improve the biological activity, such as to promote the growth and differentiation of osteoblasts. In what follows, PA6/CeO_2 biomedical composite materials are prepared by combining PA6 with modified nano-CeO_2 via electrospinning, the comprehensive properties of composite materials are explored, and the biological safety of nano-CeO_2 on composite materials is also investigated. Moreover, we systematically characterized the content of CeO_2 on the diameter, crystallinity and mechanical properties, and cell proliferation of PA/CeO_2 composite fibers. It is expected to provide theoretical and experimental basis for the practical application of PA6/CeO_2 biomedical composites.

1.1. Experimental. Granules of PA6, purchased from Dongguan Qiyuan Plastic Material Co., Ltd., China, and the modifying agent CeO_2 were used to prepare the spinning solution. Among them, the modified CeO_2 was obtained by coprocessing with CeO_2 (purchased from Leshan Wolaixi Electronic Materials Co., Ltd., China), KH550 coupling reagent, and absolute ethyl alcohol. PA6 with different concentrations of modified CeO_2 with 0, 3, 5, 7, and 9 wt.% were used to prepare the composite nanofibers. A single solvent, formic acid (analytical grade, Tianjin Yongda Chemical Reagent Co. Ltd., China) was used to prepare the polymer solution. PA6/CeO_2 nanofibers were electrospun in 80% formic acid. A high-voltage power supply of 20 kV was supplied to the syringe microtip to electrospin the nanofibers. The tip-to-collector distance was kept at 18 cm. Polymer solution was placed to the 5 mL syringe with a plastic microtip. Finally, the PA6/CeO_2 nanofibers were vacuum dried at 80°C for 24 h to remove the residual solvent, and then, the nanofibers were used for further characterizations.

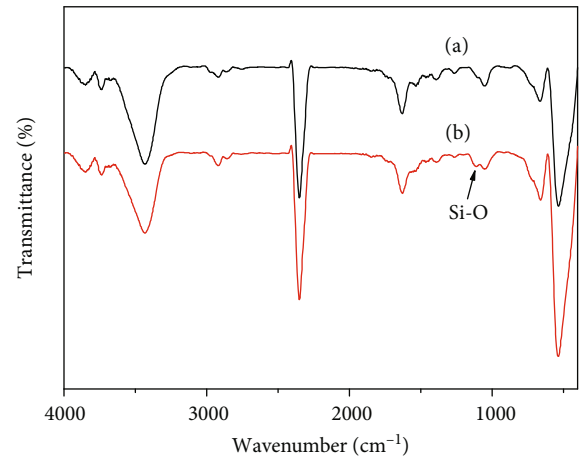


Figure 1: FT-IR spectroscopy of (a) unmodified CeO_2 and (b) modified CeO_2 with coupling agent KH550.

The morphology of the PA6/CeO_2 nanofibers was observed by using field-emission scanning electron microscopy (FESEM, S-4800, Hitachi, Japan). Then, the diameters of composite fibers from the SEM images were calculated by a statistics software. The phase composition of electrospun textile sheets (1 cm × 1 cm) has been analyzed by X-ray diffraction (XRD, D/MAX 2500, Rigaku, Japan). The bonding configurations of the nanofibers and modification effect were characterized by means of Fourier transform-infrared (FT-IR, VERTEX 70, Bruker, Germany) and EDS analysis. Tensile strength characterizations were performed for the electrospun PA6/CeO_2 nanofibers by electronic universal tensile testing machine (AGS-X, Shimadzu, Japan) to measure the strength of the fibers under external force.

The cell proliferation effect of CeO_2 on PA6/CeO_2 nanofiber membrane was tested by mouse macrophages and osteoblasts. In order to observe the manner of cell attachment on composite nanofibers, chemical fixation of cells was carried out in PA6 and PA6/5 wt.% CeO_2 samples. After a series of cell culture and incubation, the absorbance value was measured by ELISA, and finally, the samples were prepared for the cell morphology observation by SEM.

2. Results and Discussion

2.1. FT-IR Spectroscopy of PA6/CeO_2 Composite Fibers. FT-IR spectroscopy was used to study the changes of the functional groups of modified CeO_2 with coupling agent KH550 and unmodified CeO_2, as shown in Figure 1.

It can be seen that the wide peak around 3400 cm^{-1} is the absorption peak generated by the stretching vibration of water molecules. Moreover, the absorption peak of Si–O and C–H bonds appear at 1118 and 2923 cm^{-1}, respectively, in Figure 1(b) in the modified CeO_2. Therefore, it can be proven that the coupling agent KH550 was successfully coated on the surface of nano-CeO_2.

EDS measurement was used to confirm if the coupling agent KH550 coated on the surface of nano-CeO_2, as shown in Figure 2. It can be seen from Figure 2(b) that the small

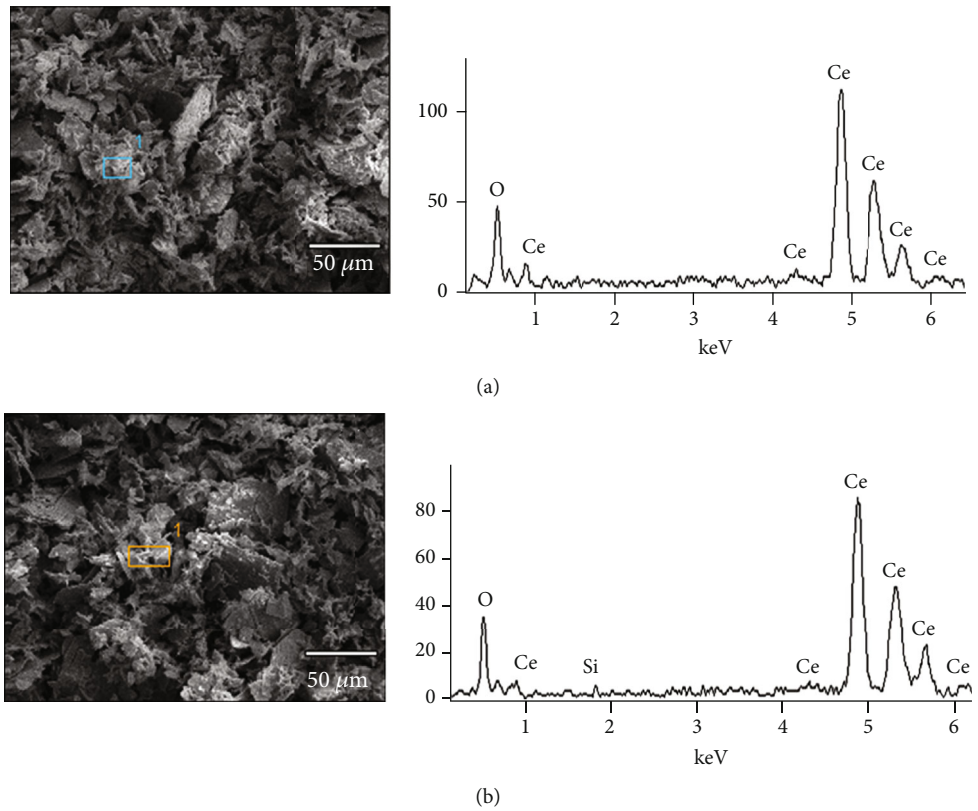


Figure 2: SEM and EDS diagrams of (a) unmodified CeO_2 and (b) modified CeO_2 with coupling agent KH550.

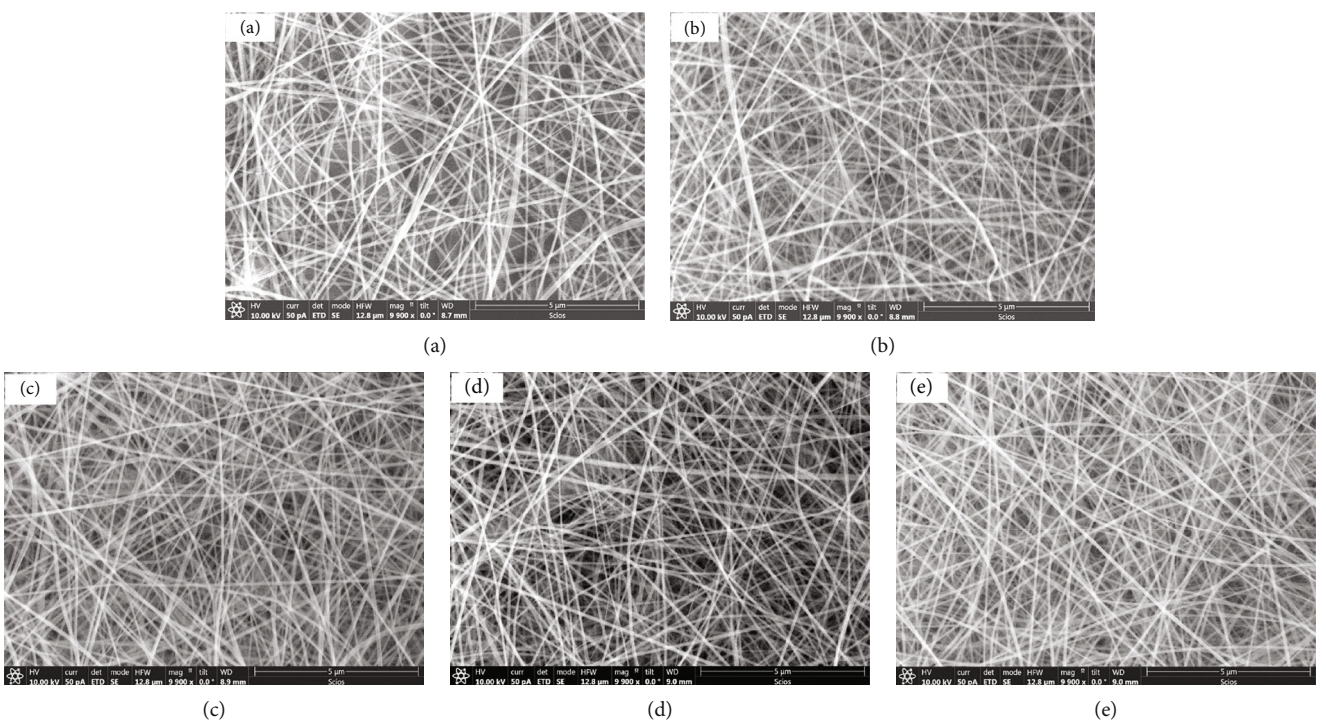


Figure 3: SEM images of composite fibers with the different concentrations of CeO_2: (a) 0 wt.%, (b) 3 wt.%, (c) 5 wt.%, (d) 7 wt.%, and (e) 9 wt.%.

Table 1: The diameter of composite fibers with the different CeO_2 concentrations.

Fibers samples	0 wt.% CeO_2	3 wt.% CeO_2	5 wt.% CeO_2	7 wt.% CeO_2	9 wt.% CeO_2
Average diameter (nm)	430	400	370	410	450
Standard deviation	11.0	11.99	8.69	16.1	17.2
Maximum (nm)	640	700	520	720	760
Minimum (nm)	280	190	160	180	160

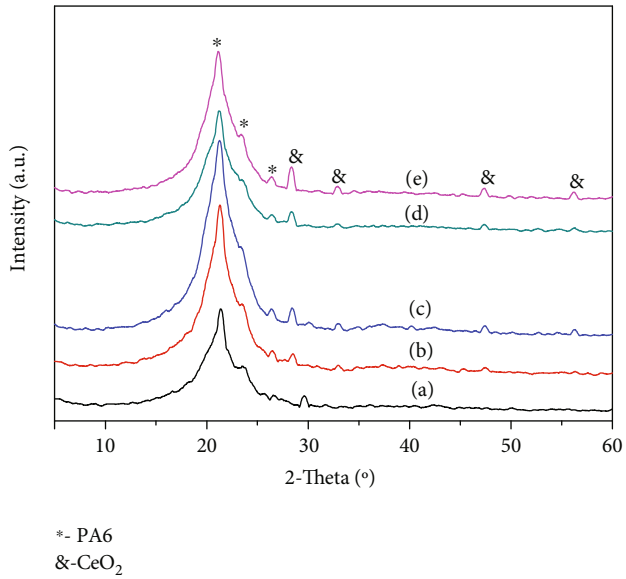


Figure 4: XRD patterns of PA6/CeO_2 composite fibers with the different concentrations of CeO_2: (a) 0 wt.%, (b) 3 wt.%, (c) 5 wt.%, (d) 7 wt.%, and (e)9 wt.%.

peak appearing at around 1.85 KeV is a characteristic peak of the Si element in the silane coupling agent, so it can also be proven that the KH550 coupling agent is successfully grafted on the surface of the nano-CeO_2, while the rate is limited.

2.2. Morphology of PA6/CeO_2 Composite Fibers. The effect of modified CeO_2 on PA6 nanofiber is investigated, and Figures 3(a)–3(e) show the SEM images of electrospun PA6/CeO_2 nanofibers for the different concentrations of modified CeO_2 with 0, 3, 5, 7, and 9 wt.%, respectively. The electrospun fibers in Figures 3(a)–3(e) all exhibit a smooth surface and uniform diameters along their lengths, and there are no obvious difference with the change of CeO_2 concentration. Then, the diameters of composite fibers from the SEM photos were calculated by a statistics software, as shown in Table 1. It shows that the average diameter of composite fibers first decreases and then increases with the increase of CeO_2 content, reaching the minimum value of 370 nm when the content of CeO_2 is 5 wt.%.

The reason for this phenomenon may be that when the content of CeO_2 increases from 0 wt.% to 5 wt.%, the concentration of solution increases and the charge density on the jet surface increases, leading to more charges on the jet, thus increasing the tensile force and decreasing the diameter of the fiber. However, when the content of CeO_2 increased from 7 wt.% to 9 wt.%, the diameter of composite fibers increased. It may be that the viscosity is too high, which makes the fibers entangled with each other, leading to a decrease in splitting ability and an increase in diameter. Thus, when the content of CeO_2 is 5 wt.%, the fiber diameter is the minimum, and the change of fiber diameter is also the smallest.

Table 2: Crystallinity of PA6/CeO_2 composite fibers with the different concentrations of CeO_2.

Fibers samples	Crystallinity
0 wt.% CeO_2	58.48%
3 wt.% CeO_2	52.07%
5 wt.% CeO_2	47.42%
7 wt.% CeO_2	45.77%
9 wt.% CeO_2	41.91%

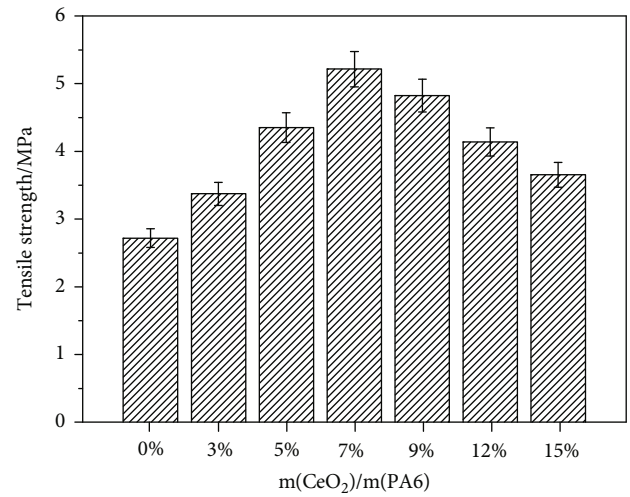


Figure 5: Tensile properties of PA6/CeO_2 composite fibers with the different concentrations of CeO_2.

2.3. The Crystallinity of PA6/CeO_2 Composite Fibers. In order to investigate the effect of the concentration of CeO_2 on the crystalline structures of PA6, the PA6/CeO_2 composite fiber film was studied by XRD, and the result is shown in Figure 4. The diffraction pattern of PA6 nanofibers exhibited a broad peak which appeared at $2\theta = 21.42°$ and two weak peaks at 23.53° and 26.37° corresponding to the characteristic of the PA6. However, the XRD data of PA6/CeO_2 nanofibers were a little weak to provide a significant result, only several weak characteristic peaks of CeO_2 appeared at 28.29°, 32.87°, 47.26°, and 56.34°. In addition, the crystallinity of composite fibers with different CeO_2 concentrations is listed in Table 2.

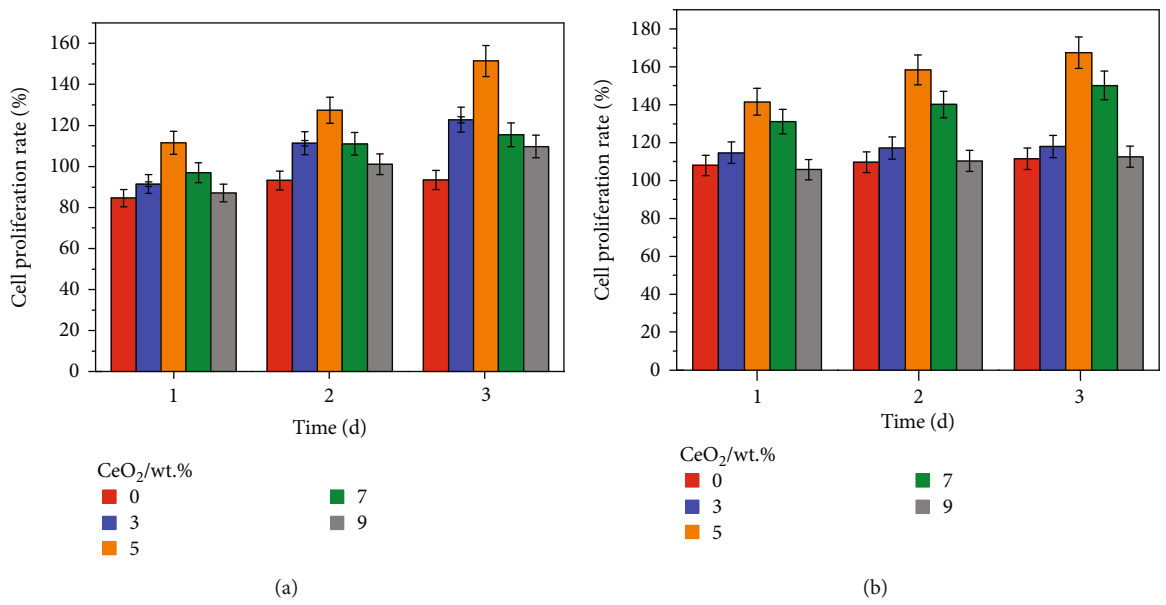


Figure 6: Cell growth measurement on (a) macrophages and (b) osteoblasts of electrospun PA6/CeO_2 nanofibers with CeO_2 concentrations of 0 wt.%, 3 wt.%, 5 wt.%, 7 wt.%, and 9 wt.%.

As can be seen from Table 2, the crystallinity of composite fibers decreases after the addition of CeO_2 and declines continually with the increase of CeO_2 content. When the content of CeO_2 was 9 wt.%, the crystallinity was the lowest, 41.91%. This phenomenon may be due to the agglomeration of nanoparticles that occurs with the excessive content of CeO_2, which disturbs the regular arrangement of macromolecules and reduces the crystallinity of composite fibers.

2.4. Tensile Properties of PA6/CeO_2 Composite Fibers. In order to investigate the effect of CeO_2 content on mechanical properties of PA6/CeO_2 composite fibers, tensile properties were conducted by an electronic universal tensile testing machine. The result is shown in Figure 5.

It can be seen from Figure 5 that the tensile strength of fibers first increases with the increased concentration of CeO_2. The reason for this phenomenon may be that the modified nano-CeO_2 is conducive to the bonding between inorganic and organic substances, so the tensile strength increases. The tensile strength of PA6 composite fibers with 7 wt.% CeO_2 was the highest, reaching 5.22 MPa, which increased by 91.84% than that of the pure PA6 fibers. However, the tensile strength decreases after too much CeO_2 is added, because excessive nano-CeO_2 would become the stress concentration point in PA6 fibers, and then, the tensile strength would decline, leading to the fracture of fibers easily.

2.5. The Proliferation of Mouse Macrophages and Osteoblasts on PA6/CeO_2 Composite Fibers. The effects of CeO_2 content on the proliferation of mouse macrophages and osteoblasts were tested by cell experiments. Figure 6 shows the quantitative cell viability test results.

According to Figure 6, the cellular proliferation gradually increases from 1 day to 3 days, and also, the groups with the addition of CeO_2 was higher than those in the blank group, indicating that PA6/CeO_2 electrospinning fibers with CeO_2 were non-toxic to macrophages and osteoblasts and has good biocompatibility. Meanwhile, it can also prove that PA6/CeO_2 electrospinning fibers can promote cell proliferation.

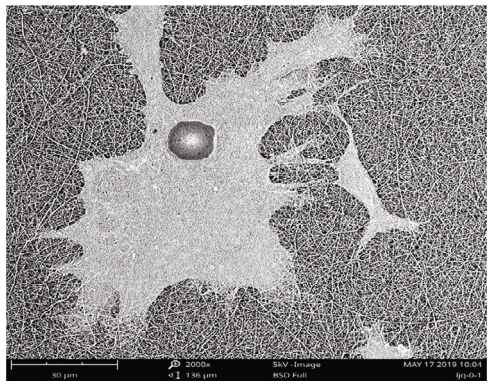
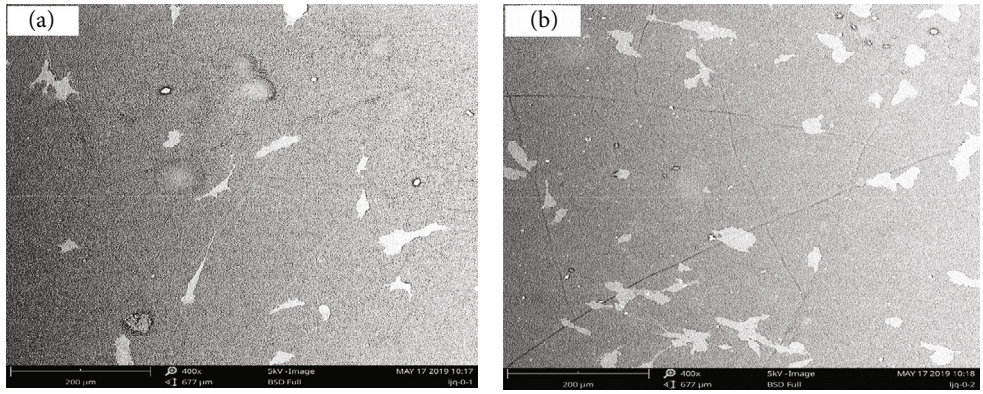


Figure 7: SEM image of mouse osteoblast cultured on PA6/5 wt.% CeO_2 composite nanofibers for 1 day.

Compared with Figures 6(a) and 6(b), it can be seen that the proliferation capacity of CeO_2 for osteoblasts is greater than that of macrophages, and when the content of CeO_2 is 5 wt.%, PA6/CeO_2 electrospinning fibers have the largest ability to promote the proliferation of osteoblasts. By 3 days of culture, the cell proliferation rate reached 167%.

In order to investigate the statistical significance of the cell proliferation effect of cerium oxide, SPSS software was used to analyze the significant difference of the test data in Figure 6, and the results showed that all the PA6/CeO_2 nanofibers from 1 day to 3 days with different CeO_2 concentrations have a significant difference compared with the blank PA6 fiber.

2.6. Morphology of Mouse Osteoblast on PA6/5 Wt.% CeO_2 Composite Nanofibers. Figure 7 shows the SEM image of



(a) (b)

Figure 8: SEM images of osteoblasts cultured on (a) pure PA6 fibers, and (b) PA6/5 wt.% CeO_2 composite fiber membrane.

mouse osteoblast cultured on PA6/CeO_2 nanofibers for 1 day. It can be seen that the nucleus of the cell on the fiber membrane is round, which grows along the direction of the fiber on the nanofiber membrane, and it can be clearly seen that the cell has been completely or partially embedded in the fiber interior, and the surface of the membrane is basically completely covered by the cell. Therefore, osteoblasts grow successfully and well in PA6/CeO_2 composite nanofiber membranes.

Figure 8 shows the SEM images of osteoblasts cultured on pure PA6 fibers and PA6/CeO_2 fibers membrane for 1 day. Obviously, the number of cells in the PA6/5 wt.% CeO_2 fiber membrane is significantly higher than that in the pure PA6 fiber membrane. As the scaffold itself, PA6 nanofibers play a role in guiding cell growth, and the number of cells increases significantly after the addition of CeO_2, so CeO_2 can significantly promote the proliferation of CeO_2 osteoblasts.

3. Conclusions

CeO_2 blended in PA6 composite fibers with smooth surface and uniform diameters is successfully prepared by an electrospinning process. High aspect ratio PA6/CeO_2 composite nanofibers with diameters of about 370 to 450 nm were bound in all fibers. It shows that the CeO_2 has no obvious effect on the morphology of PA6 fibers. With the increase of CeO_2 content, the diameter of composite fibers first decreases and then increases, reaching the minimum value of 370 nm at 5 wt.%. The tensile strength of the fibers first increases and then decreases with the increase of CeO_2 content, reaching the best value of 5.22 MPa at 7 wt.%. The crystallinity of PA6 composite fibers decreases after the addition of CeO_2 and declines continually with the increase of CeO_2 content.

PA6/CeO_2 electrospinning membrane is nontoxic to macrophages and osteoblasts and has good biocompatibility. The proliferation capacity of CeO_2 to osteoblasts is greater than that of macrophages, and when the content of CeO_2 is 5 wt.%, PA6/CeO_2 electrospinning fibers have the largest ability to promote the proliferation of osteoblasts. Current results have brought a deeper insight into the formation of electrospun nanofibers in the presence of a modifying agent CeO_2 and show the pitfalls and benefits in biomedical aspects.

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Fig. 1: FT-IR spectroscopy of (a) unmodified CeO2 and (b) modified CeO2 with coupling agent KH550. Figure 4: XRD patterns of PA6/CeO2 composite fibers with the different concentrations of CeO2: (a) 0 wt.%, (b) 3 wt.%, (c) 5 wt.%, (d) 7 wt.%, and (e) 9 wt.%. Figure 5: tensile properties of PA6/CeO2 composite fibers with the different concentrations of CeO2. Figure 6: cell growth measurement on (a) macrophages and (b) osteoblasts of electrospun PA6/CeO2 nanofibers with different CeO2 concentrations of 0 wt.%, 3 wt.%, 5 wt.%, 7 wt.%, and 9 wt.%. *(Supplementary Materials)*

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