Eurasian J. Bio. Chem. Sci. 1(2): 54-58, 2018

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.gov.tr/ejbcs

Fabrication and characterization of PCL/ZnO-NP nanocomposite for wound dressing applications

Alev Akbaş [1]*, Melek Erol Taygun [1], Sadriye Küçükbayrak [1]

[1] *Istanbul Technical University, Chemical & Metallurgical Engineering Faculty, Chemical Engineering Department, İstanbul, Turkey*

*Corresponding author : akbas16@itu.edu.tr
Orcid No: 0000-0002-4092-8474

Abstract: Nanotechnology has a critical role in biotechnology and medicine with aiming to develop portable, low cost, safe and practical technologies. One of these technologies includes construction of three dimensional biomimetic nanofiber scaffolds with using electrospinning method. Nanofibers have started to be used with the development of nanotechnology in tissue engineering because of its similarity to natural human tissues. The architecture of original extracellular matrix at nanoscale level can be mimicked by these scaffolds. Meanwhile, metal nanoparticles are also used in tissue engineering because of their unique features such as optical, electronic, catalytic, and antibacterial. Zinc oxide (ZnO) is a transition metal oxide and it has good catalytic, electrical, photochemical, optical, antibacterial, enhanced cell proliferation and wound healing properties. Zn ion also acts as regulator for auto debridement and keratinocyte migration, both of which are essential for wound repair. Polycaprolactone (PCL) which is biocompatible and biodegradable synthetic polymer used as biomaterial for various biomedical applications such as tissue engineering scaffolds and wound dressings. In the present study, zinc oxide nanoparticles (ZnO-NPs) synthesized by microwave irradiation were used for the fabrication of PCL/ZnO-NP nanocomposite via electrospinning method. The effects of the ZnO nanoparticle concentration on the fiber diameter and fiber morphology were investigated using a scanning electron microscope (SEM). The presence of ZnO-NPs in the structure was determined by X-ray diffraction (XRD). It was observed that the average diameter of nanofibers was below micrometers. Overall results showed that PCL/ZnO-NP nanocomposite were found to be suitable for wound dressing applications.

Keywords: ZnO nanoparticle, PCL, nanofiber, microwave irradiation, wound dressing, antibacterial.

1. Introduction

Nanotechnology is an emerging field of science which deals with synthesis and development of materials at the scale of nanometer. Recent advancements in the field of nanotechnology opened new channels for its applications in biomedical area. Tissue engineering is one of the biomedical applications and is an interdisciplinary field to build biological substitutes that restore or improve the function of injured tissue (Liu et al., 2017). Tissue engineering is used to fabricate functional constructs, scaffolds, which are ranging from one dimension (1D) to three dimension (3D) structures. Scaffolds play an important role for the formation of the extracellular matrix (ECM) and restore damaged tissues via cell proliferation and migration (Augustine et al., 2014). Among the various scaffold fabrication techniques, electrospinning presents substantial advantages to be able to obtain uniform and continuous nanofibers and fabrics with adjustable pore structure and diameters ranging from nanometers to micrometers from synthetic (PCL, PGA, PLGA, PVA) and many natural (collagen, gelatin, chitosan, fibrin) polymers. The nanofibers with adjustable pore structure and large surface to volume ratio have been used in many fields particularly biomedical applications such as tissue scaffold, wound dressing and drug delivery (Liu et al., 2017).

Wound dressing materials act as physical barriers to maintain permeability for moisture and oxygen and to protect the wound mainly against microorganisms (Mogoşanu et al., 2014). A standard wound dressing material should be non-toxic and have good permeability as well as skin flexibility, biocompatibility and biodegradability characteristics. In contrast to traditional cotton-gauge dressings which do not adequately meet the requirements of wound care, fiber mats fabricated by electrospinning technique have potential to provide an excellent platform for wound healing. Nanofibers fabricated by electrospinning process have high surface area to volume ratio, high porosity and gas permeability. These properties provide to accelerate wound healing by increasing in the rate of gas permeability for cell, skin regeneration , moisture regeneration, removal of exudates and hemostasis (Rath et al., 2016). Wound dressings that are impregnated with antimicrobials (Ag nanoparticles (Wei et al., 2016), ZnO nanoparticles (ZnO NPs) (Augustine et al., 2014)), growth

factors, collagen or enzyme debriding agents can accelerate wound healing and show antimicrobial properties (Mogoşanu et al., 2014).

Zinc oxide (ZnO) is a unique material with a wide band gap of 3.37 eV and a large excitation binding energy (60 meV) (Faal Hamedani and Farzaneh, 2006). ZnO is currently listed as a 'generally recognized as safe' (GRAS) material by the Food and Drug Administration and is used as a food additive (Augustine et al., 2014). It is generally utilized in many dermatological applications including sunscreens, and skin care products and enhanced healing of wounds (Shoja et al., 2015). Zinc deficiency extends the healing period of wounds. ZnO NPs are one of the best appropriate source for wound applications by enhancing re-epithelialization, reducing inflammation and bacterial growth. Zinc being the cofactor of metaloprotein plays an important role in the regeneration of ECM. Moreover, Zinc also acts as regulator for auto debridement and keratinocyte migration, both of which are essential for wound repair (Landsdown et al., 2007). ZnO NPs can improve cell adhesion, proliferation and cell migration through growth factor mediated pathways by causing reactive oxygen species (ROS) production. (Augustine et al., 2014; Toduka et al., 2012; Premanathan et al., 2011). The ZnO NP can be synthesized by using various techniques such as hydrothermal, sol–gel, precipitation, microemulsion, chemical vapor deposition and solid state reaction (Król et al., 2017). The utilization of microwave irradiation as a heating method in the synthesizing of nanoparticles has the advantages of producing small particle size with high purity due to short reaction time compared to the conventional methods (Faal Hamedani and Farzaneh, 2006). PCL which is non-toxic, biocompatible, biodegradable and synthetic aliphatic polyester in biomedical applications can be used in drug delivery devices, tissue engineering scaffolds and wound dressings (Augustine et al., 2014; Shoja et al., 2015, Liu et al., 2017). In the present study, ZnO NPs synthesized by microwave irradiation were used for the fabrication of PCL/ZnO-NP nanocomposite via electrospinning method.

2. Materials and Method

2.1. Materials

Polycaprolactone (PCL, Mn = 70,000–90,000) and sodium hydroxide (NaOH) were obtained from Sigma–Aldrich Chemicals. Glacial acetic acid (AA), formic acid (FA), zinc nitrate hexahydrate (Zn $(NO_3)_2$ $6H_2O$) and soluble starch were purchased from Merck. All the reagents were of analytical grade and they were used without further purification.

2.2. Preparation of ZnO nanoparticles

The ZnO NPs were synthesized by the microwave method using Zn $(NO_3)_2$ $6H_2O$ and NaOH as precursors and soluble starch as a stabilizing agent. Starch was dissolved in distilled water at 87°C. 10 ml, 1M of Zn $(NO_3)_2$ $6H_2O$ solution was added to the starch solution. The obtained solution was stirred continuously using the magnetic stirrer for 15 minutes until complete dissolution occurs. Subsequently, 2M NaOH solution was added drop by drop to adjust pH= 7 of the solution. By the addition of NaOH the aqueous clear solution turned into a milky white without any precipitation. The solution was homogenized by using a Hielscher UP200 HT ultrasonic homogenizer before microwave treatment to improve nanoparticle distribution. Following this step, the solution was placed under microwave irradiation by using a domestic microwave oven operated at 600 W and 2 minutes with a frequency of 2450 MHz. After the microwave irradiation, the solution was centrifuged. In order to remove the byproducts and excessive starch bound to the nanoparticles, the precipitate was washed with distilled water twice. The powder of the ZnO nanoparticles was obtained after drying at 150°C.

2.3. Preparation of PCL/ZnO-NP nanocomposite

PCL membranes containing ZnO NPs and neat PCL were prepared by electrospinning using 15 wt. % PCL in co-solvent of AA:FA (ratio of 1:1 v/v). ZnO NPs were first dispersed in a co-solvent of AA:FA at room temperature for overnight to be able to fabricate PCL/ZnO-NP nanocomposite membrane containing 10 wt.% ZnO NPs (relative to the total polymer weight). Then, a certain amount of PCL was added into the dispersion containing ZnO NPs and stirred at room temperature for overnight in order to obtain homogenous solution. PCL solution stirred at room temperature for 3 hours for the fabrication of neat PCL membrane.

Nanofibers were fabricated by using a Nanospinner 24 Touch, Inovenso Co. In the electrospinning process, the tip to collector distance was maintained at 21 cm and an electric voltage of 24 kV was applied. The feed rate of the solution was precisely controlled by a syringe pumping system which was adjusted to a flow rate of 1.5 ml h^{-1}. The electrospun nanocomposite fibers were accumulated as nonwoven mats on a grounded target wrapped with aluminum foil. All electrospinning experiments were carried out at ambient conditions.

2.4. Characterization

Scanning electron microscope (SEM) (QUANTA FEG 250) was used to measure the nanoparticle size and determine the nanoparticle shape. The surfaces of the ZnO NPs and PCL/ZnO-NP nanocomposite fibers were sputter coated (SC7620 sputter coater, Quorum Technologies Ltd, United Kingdom) with platinum for 120s prior to SEM investigations. For each experiment, the average nanoparticle and nanofiber diameters were analyzed by the help of an image visualization software (Image-J, National Institute of Health, USA) from about 50 measurements of the random nanoparticles and nanofibers. X-ray Diffraction (XRD-PANalytical XPert Pro) analysis was conducted to prove ZnO NPs production and the presence of ZnO NPs in the PCL nanofiber. The functional groups of the ZnO NPs and the PCL nanocomposite fibers were determined by using a Fourier-transform infrared (FT-IR) spectroscopy. FT-IR spectra were collected using a spectrometer (Spectrum 100, Perkin Elmer) with 4 cm^{-1} resolution in transmittance mode in the mid-IR region (4000–650 cm^{-1}). The in vitro studies were carried out in a phosphate buffered saline (PBS) solution for time periods of 1, 2, 4 and 7 days

to determine the amount of Zn ion release from PCL/ZnO-NP nanocomposite. Fabricated nanocomposite were cut to 1x1 cm^2 area and were placed in a 10 ml PBS solution (pH=7.4). The release studies were carried out at 37 °C. The amount of Zn ions present in the PBS was determined by using a inductively coupled plasma–mass spectrometer (ICP-MS, Perkin Elmer Optima 2100 DV model).

3. Results

SEM investigations were conducted on nanoparticles and nanofibers to evaluate the morphology and size distribution of them. Representative morphological features and size of the ZnO NPs, neat PCL nanofiber and PCL/ZnO-NP nanocomposite can be seen from Figure 1. SEM images indicated that ZnO NPs were spherical in shape with an average diameter of 268±95 nm (Fig. 1 (a)). SEM micrographs, given in Fig. 1(b), revealed that the electrospun PCL nanofiber mats were composed of randomly oriented, uniform, and bead free nanofibers, with an average fiber diameter of 479±170 nm. On the other hand, nanocomposite fiber mats were also successfully generated without any beads through the electrospinning process (Figs. 1(c) and (d)). The average diameter of PCL/ZnO-NP nanocomposite fiber mat is 372±198 nm. ZnO NPs also can be seen clearly from Figs. 1(c) and (d). These results indicated that the introduction of ZnO NPs into the PCL nanofiber decreased the fiber diameter.

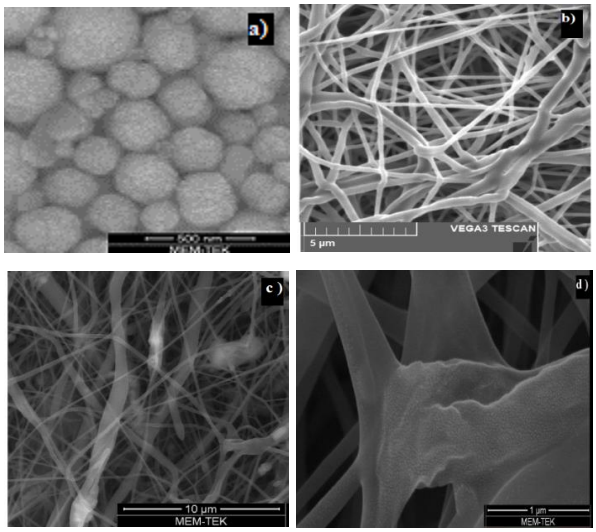

Figure 1. SEM images of the ZnO NPs (a), neat PCL nanofiber (b) and PCL/ZnO-NP nanocomposite (c and d)

The XRD patterns of the ZnO NPs and PCL/ZnO-NP nanocomposite were given in Figure 2. As can be seen from Fig. 2(a), peaks at 2θ = 32.25°, 34.87°, 36.77°, 47.99°, 57.26°, 63.27°, 66.75° and 68.24° confirmed the formation of ZnO NPs (Zhang et al., 2011). The peaks at 2θ =33.47° and 36.65° which can be seen from Fig. 2(b) show the presence of ZnO NPs in the PCL nanofiber. Moreover, the clear and sharp diffraction peaks at 2θ=21.92° and 24.23° are evidence of semi-crystalline nature of neat PCL (Augustine et al., 2014). XRD studies revealed that ZnO-NPs were successfully incorporated into the PCL neat.

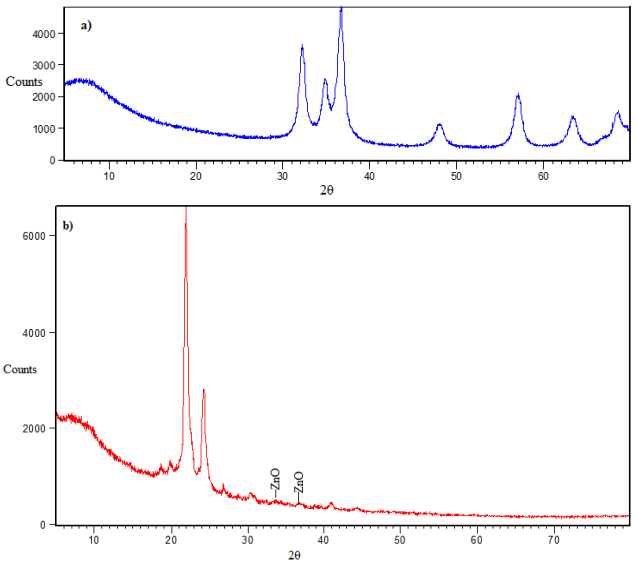

Figure 2. XRD patterns of the ZnO NPs (a) and PCL/ZnO-NP nanocomposite (b).

Figure 3 shows release amount of zinc ions from PCL/ZnO-NP nanocomposite to PBS for the time periods of 1, 2, 4 and 7 days. Results showed that the release of zinc ions was between 0.126–0.224 µg/ml. Possible burst release and higher concentration of zinc could be cytotoxic and provide unfavorable conditions for cell attachment and growth. Therefore, it is proposed that the slow release of zinc, as observed in PCL/ZnO-NP nanocomposite, is favorable for the possible tissue engineering applications of the present sample.

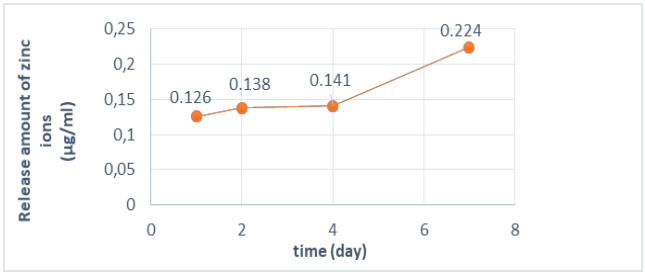

Figure 3. Release amount of zinc ions from the PCL/ZnO-NP nanocomposite in PBS solution for time periods 1, 2, 4 and 7 days.

FT-IR spectra of ZnO NPs which indicates the presence of starch in the structure of nanoparticles can be seen from Figure 4 (a). The broad absorption peaks at about 3382 and 1645 cm^{-1} are due to the hydroxyl groups of chemisorbed and/or physisorbed H_2O molecules on the particle surface. The peak at 1153 cm^{-1} was attributed to C-O bond stretching of the C-O-H group, and the peak at 1083 cm^{-1} was ascribed to C-O bond stretching of the C-O-C group in the anhydroglucose ring of starch (Zhang et al., 2011). Figure 4 (b) shows FT-IR spectra of the neat PCL nanofiber and PCL/ZnO nanocomposite. The peaks at PCL related stretching modes are represented by the peaks at 2945 cm^{-1} (asymmetric CH_2 stretching), 2867 cm^{-1} (symmetric CH_2 stretching), 1722 cm^{-1} (C=O stretching), 1294 cm^{-1} (C–O and C–C stretching), and 1238 cm^{-1} and 1164 cm^{-1} corresponding to C–O–C

asymmetric and symmetric stretching vibrations (Gomes et al., 2015; Xue et al., 2015). The PCL/ZnO-NP nanocomposite and neat PCL nanofiber presented the same spectra in the wave number range of 700–3.100 cm^{-1}.

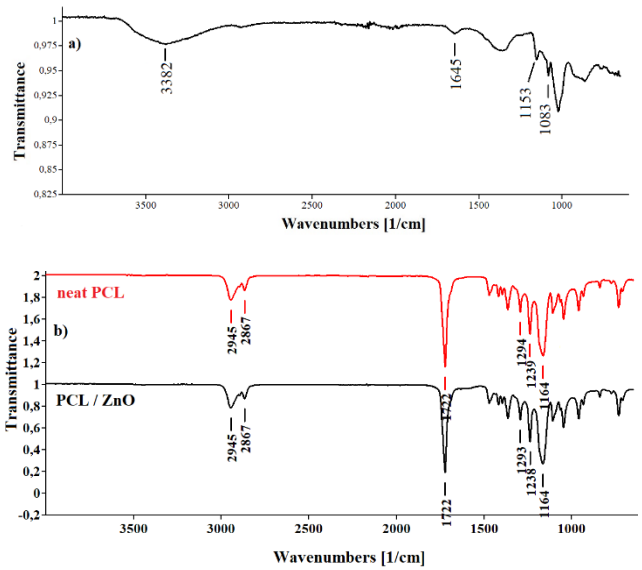

Figure 4. FT-IR spectra of the ZnO NPs (a), FT-IR spectra of the neat PCL nanofiber and PCL/ZnO-NP nanocomposite (b)

4. Discussion

ZnO NPs were successfully synthesized by microwave assisted hydrothermal method with short reaction time, low cost and very simple compared to the conventional methods. Using the water as solvent in the synthesis of ZnO nanoparticles resulted to an immediate agglomeration due to the high polarity of water. The presence of starch in the reaction medium provides to produce nanoparticles in spherical shape and helps to reduce the agglomeration (Hasanpoor et al., 2015; Zhang et al., 2011).

SEM image of neat PCL nanofiber indicated that the fibers had smooth surface. The surface of PCL/ZnO-NP nanocomposite was rough due to the agglomeration of ZnO NPs. SEM images of neat PCL nanofiber and PCL/ZnO-NP nanocomposite indicated that ZnO NPs in the fibers caused to decrease of the fiber diameter. It was thought that the reason of the decreasing the diameter of the fibers containing ZnO NPs was that the addition of the ZnO NPs into spinning solution leads to increase in the electrical conductivity of the solution. The increase in conductivity of the solution can increase the electric charge density on the surface of the ejected spinning jet. Thus, the increasing charge density of spinning jet can decrease self-repulsion tension and increase elongation forces. As a result of this, higher elongation forces could provide to overcome the self-repulsion tension during the electrospinning process and the diameters of the fabricated fibers can decrease. (Augustine et al., 2014; Münchow et al., 2015).

It was reported that upon the dissolution of the polymeric matrix, the controlled release of therapeutic ions brings about additional functionalities, including osteogenesis, angiogenesis, and antibacterial effects. Therefore, it is of great importance to determine the amount of therapeutic ions released from the polymeric matrix. However, high concentrations of these ions can cause free radical formation and cytotoxicity. Thus, it is necessary to control the release of zinc ions at a clinically acceptable rate. Lee et al., who studied dose-dependent cytotoxicities after the treatment with ZnO nanoparticles in human epidermal keratinocyte HaCaT cells stated that the amount of zinc ions released from ZnO nanoparticles (~20 nm, spherical shape and hydrodynamic size were 986±46 nm) as 10, 20, 40 and 80 µg/mL for the time periods of 6, 12 and 24 h. At the dosage of 10 µg/mL, approximately 100% cell viability was observed for 24 h. Although, at the dosage of 20 µg/mL, viability of cell decreased with the exposure time dependence, viability of cell was found to be greater than 50% for 24 h. At 40 and 80 µg/mL doses, significant differences in cell viability were observed below 50% for 24 h (Lee et al., 2012). In our study, the release amount of zinc ions was below than the values in that study. PCL/ZnO-NP nanocomposite can be considered as non-toxic to the skin.

It was reported in the literature that ZnO NPs displayed antibacterial activity and enhanced cell proliferation/wound healing (Augustine et al., 2014; Münchow et al., 2015). As mentioned before, ZnO NPs serve as a sustained ionic Zn source best suited for topic wound application by enhancing re-epithelialization, decreasing inflammation and bacterial growth. It is considered that the fabricated material can be used as a wound dressing material due to ZnO NPs were successfully incorporated into the PCL fiber and the release of zinc ions from the PCL/ZnO-NP nanocomposite has been observed.

5. Conclusions

In this study, ZnO NPs and electrospun PCL scaffolds incorporated with ZnO NPs were successfully fabricated and characterized. SEM and XRD analysis revealed the presence of ZnO NPs in the fibers. These findings indicated that the currently described electrospun nanocomposite fiber mats are very promising materials as they combine the high biocompatability of PCL, the beneficial effects of zinc ions on antibacterial properties and an interconnected porous structure of electrospun nanofibers that may allow cell adhesion, cell invasion and vascularization. It is believed that the PCL nanocomposite mat incorporated with the wound healing and antibacterial properties provided by ZnO NPs has potential to be used in wound dressing applications.

Acknowledgements

This work was supported by Istanbul Technical University Scientific Research Office (BAP). Project number is MYL-2018-41501.

References

Augustine R, Malik HN, Singhal DK, Mukherjee A, Malakar D, Kalarikkal N, Thomas S 2014. Electrospun polycaprolactone/ZnO nanocomposite membranes as biomaterials with antibacterial and cell adhesion properties. Journal of Polymer Research, 21(347): 1-18.

Faal Hamedani N, Farzaneh F 2006. Synthesis of ZnO Nanocrystals with Hexagonal (Wurtzite) Structure in Water Using Microwave Irradiation, Journal of Sciences, Islamic Republic of Iran 17(3): 231-234.

Gomes SR, Rodrigues G, Martins GG, Roberto MA, Mafra M, Henriques CMR, Silva JC 2015. In vitro and in vivo evaluation of electrospun nanofibers of PCL, chitosan and gelatin: A comparative study, Mater. Sci. Eng. C 46:348–358.

Hasanpoor M, Aliofkhazraei M, Delavari H 2015. Microwave-assisted synthesis of zinc oxide nanoparticles, Procedia Materials Science 11:320 – 325.

Król A, Pomastowski P, Rafińska K, Railean-Plugaru V, Buszewski B 2017. Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism, Advances in Colloid and Interface Science 249:37–52.

Lansdown ABG, Mirastschijski U, Stubbs N, Scanlon E, Agren MS 2007. Zinc in wound healing: theoretical, experimental, and clinical aspects, Wound Repair Regen. 15(1): 2-16.

Lee SH, Lee R, Kim Y, Kim M 2012. Toxic Response of Zinc Oxide Nanoparticles in Human Epidermal Keratinocyte HaCaT Cells, Toxicol. Environ. Health. Sci 4(1): 14-18.

Liu M, Duan X, Li Y, Yang D, Long Y 2017. Electrospun nanofibers for wound healing. Materials Science and Engineering C 76: 1413–1423.

Mogoşanu GD, Grumezescu AM 2014. Natural and synthetic polymers for wounds and burns dressing. International Journal of Pharmaceutics 463: 127– 136.

Münchow EA, Albuquerque MTP, Zero B, Kamocki K, Piva E, Gregory RL, Bottino MC 2015. Development and characterization of novel ZnO-loaded electrospun membranes for periodontal regeneration. Dental Materials 31: 1038–1051.

Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G 2011. Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation, Nanomedicine: Nanotechnology, Biology, and Medicine 7: 184–192.

Rath G, Hussain T, Chauhan G, Garg T, Goyal AK 2016. Development and characterization of cefazolin loaded zinc oxide nanoparticles composite gelatin nanofiber mats for postoperative surgical wounds, Materials Science and Engineering C 58: 242–253.

Shoja M, Shameli K, Ahmad MB, Kalalntarı K 2015. Preparation, characterization and antibacterial properties of Polycaprolactone/ZnO microcomposites. Digest Journal of Nanomaterials and Biostructures 10(1): 169-178.

Toduka Y, Toyooka T, Ibuki Y 2012. Flow Cytometric Evaluation of Nanoparticles Using Side-Scattered Light and Reactive Oxygen Species-Mediated Fluorescence−Correlation with Genotoxicity, Environ. Sci. Technol.46: 7629−7636.

Wei Q, Xu F, Xu X, Geng X, Ye L, Zhang A, Feng Z, 2016. The multifunctional wound dressing with core–shell structured fibers prepared by coaxial electrospinning. Front. Mater. Sci. 10(2): 113–121.

Xue J, Shi R, Niu Y, Gong M, Coates P, Crawford A, Chen D, Tian W, Zhang L 2015. Fabrication of drug-loaded anti-infective guided tissue regeneration membrane with adjustable biodegradation property, Colloids and Surfaces B: Biointerfaces 135: 846–854.

Zhang G, Shen X, Yang Y 2011. Facile Synthesis of Monodisperse Porous ZnO Spheres by a Soluble Starch-Assisted Method and Their Photocatalytic Activity, The Journal of Physical Chemistry C 115: 7145–7152.