Enhanced Antibacterial Property by the Synergetic Effect of TiO2 and ZnO Nano-Particles in Biodegradable Hydrogel

Fatma Ozge Gokmen, PhD
Sinan Temel, PhD
Elif Yaman, PhD
Bilecik Şeyh Edebali University,
Central Research Laboratory, Bilecik, Turkey

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Abstract

In this study, three types of nanocomposite hydrogel were produced by free radical polymerization and chemical bath deposition technique together. TiO2 nanoparticles (NPs) have been incorporated in polymeric matrices in order to provide antimicrobial activity to the biodegradable hydrogel. Then, ZnO NPs have been deposited on the surface of the hydrogel to improve antibacterial activity. Structural and antibacterial properties of above nanocomposites were fully determined by X-Ray diffractometer (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-Ray Spectrometer (EDX), and ASTME-2149 methods. At the reaction time of 60 minutes, nano-ZnO particles on hydrogel were fully developed. Zn content of hydrogel was 35.89 at. %, Ti content of hydrogel was also determined as 0.16 at.%. In respect to antibacterial activity tests results, Escherichia coli were more resistant to hydrogel and ZnO nanoflower than Staphylococcus aureus.

Keywords: Antibacterial activity, Nano-TiO2, Nano-ZnO, Hydrogel

Introduction

Bacteria cause infections which result in important health problem for millions of people (Shuai et al., 2017). The principle way to kill bacteria is using antibiotics. But, some bacteria can gain the resistant to commonly used antibiotics and restrict the antibiotic treatments for infectious diseases efficiencies (Hede, 2014). Nowadays, antibiotic resistance is a significant problem for public health. For this reason, new effective antibacterial material development is necessary (Teymourinia et al., 2019).

The interest is increasing within improving antibacterial polymers as antimicrobial agents for the applications in biomedical, food packaging and surgical device sterilizing area (Athanasoulia et al., 2018). Antibacterial polymers can inhibit bacteria growth, further they can kill bacteria (Wahid et al., 2019). Hydrogels are three dimensional (3D) polymers which made of both natural and synthetic materials dominating high number of flexibility. Superior swelling property of hydrogels makes them an ideal material for variable applications. Some characteristic properties of hydrogels can be listed as desired functionality, reusability, reversibility, sterilizability and biocompatibility (Ullah et al., 2015; Khan et al., 2013).

Antibacterial property of hydrogels can be enhanced with metal and metal oxide nanoparticles (NPs). Polymer matrices acts a substrate for active antimicrobial nanoparticles. Compared to other used materials, NPs have some superior characteristics such as small particle size, higher specific surface area to volume ratio and large proportion of surface atoms (Liu et al., 2018). TiO_2 is one of the promising NPs due to its unique properties like well-stability, self-cleaning, environmental-friendly and the most important property is antibacterial capability (Athanasoulia et al., 2018). When the energy supplied higher than the band gap of TiO_2, the electron (e−)/hole (h+) pairs are formed and react with O_2 and H_2O to form superoxide anion radicals ($O_2 \cdot -$) and hydroxyl radicals (·OH). h+, $O_2 \cdot$ and ·OH species are highly reactive and they provide the disruption of bacteria cell. In addition to this, TiO_2 has some disadvantages as biocidal agent due to its large bandgap energy and fast recombination rate of photogenerated electron–hole pairs (Hwang et al., 2011). Therefore, various nano-materials with different antibacterial property can be added to TiO_2 doped materials to enhanced its antibacterial property (Jia et al., 2019). Due to its high specific surface area, excellent hydrophobicity and oxidizing ability, ZnO has been widely used to inhibit the growth of microorganisms. Reducing ZnO particles to nanoscale provides them ability to kill bacteria rapidly (Liu et al., 2019).

In this case, using of both TiO_2 and ZnO NPs in polymeric substrates should be improved to enhance antibacterial property. However, there are no reports for production of hydrogel with TiO_2-NPs and ZnO deposition on this hydrogel for antibacterial applications. In this study, a novel and high efficient antibacterial nanocomposite was synthesized. Firstly, nano-TiO_2 doped hydrogel was produced and then ZnO nano-flowers were grown on the hydrogel to enhance the antibacterial property of nanocomposite material.

Materials and Methods

The chemicals used in this study are described in this section. The techniques used for preparation and characterization of nanocomposite hydrogels were also detailed in this section.

Materials

Ammonium persulfate (≥98%), *N,N'*-methylenebis(acrylamide) (99%), Titanium(IV) oxide (<100 nm, 99.99%), Zinc nitrate hexahydrate (98%) and ammonia (>99.95%) were purchased from Sigma-Aldrich (USA). The deionized water was used in all experiments.

Synthesis of Hydrogels

In the first part of the study, acrylic acid (AA) hydrogels were synthesized by free radical polymerization technique using with a radicalic initiator (ammonium persulfate) and a crosslinking agent (*N,N'*-methylenebis(acrylamide)). TiO_2 NPs were used as a dopant to produce 1 wt.% TiO_2 doped hydrogel. Experimental details were given in previous study (Temel et al., 2018). Pure and TiO_2 doped hydrogel was labelled as H and TH, respectively.

Synthesis of ZnO NPs on Hydrogels

In the second part of the study ZnO NPs on TH were deposited by chemical bath deposition technique (CBD) at different deposition times (15-30-45 and 60 min) as given in detail in previous study (Temel et al., 2019). ZnO NPs deposited TH was labelled ZTH.

Characterization

The X-ray diffractometry of ZTH was conducted by using XRD instrument (Panalytical, Empyrean) using CuKα (λ = 1.5405 Å) radiation at 40 kV with 2θ ranging from 20° to 80° and a scanning rate of 2°/min.

Attenuated Total Reflectance Fourier Transform (ATR-FTIR) spectra of H, TH and ZTH were obtained by using a Perkin Elmer Spectrum 100 spectrometer in the wave number ranging from 4000 to 380 cm^{-1}, at a resolution of 4 cm^{-1}.

Field Emission Scanning Electron Microscopy (FESEM) micrographs of H, TH and ZTH were achieved by using a ZEISS SUPRA 40 VP microscope. All samples were sputter coated with gold/palladium. The content of carbon, oxygen, titanium and zinc in H, TH and ZTH were determined by using Energy Dispersive X-Ray Spectrometer (EDX).

Antibacterial Activity Tests

The antibacterial activity tests were carried out in accordance with ASTME2149 (Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions) with the bacteria *Staphylococcus aureus (S. aureus)* ATCC 6538 and *Escherichia coli (E. Coli)* ATCC 8739.

Results and Discussion

In this section, results from the characterization of H, TH and ZTH and their antibacterial properties were presented and discussed.

Characterization of H, TH and ZTH:

XRD spectrum of H, TH and ZTH were given in Figure 1. The XRD pattern of H and TH showed a broad peak, which was proved with its amorphous feature and, intense and sharp peaks shown in the XRD pattern of ZTH demonstrate the crystalline characteristic of ZnO. The XRD diffraction pattern of the ZTH has matched with that of the hexagonal structured ZnO (ICDD data: 98-003-1052) and it has polycrystalline structure (Temel et al., 2017).

In Figure 2, FTIR spectrum of H, TH and ZTH were demonstrated. Characteristic peaks which belongs to acrylic acid were located at 2942 cm^{-1}, 1693 cm^{-1} (C=O), 1452 cm^{-1} (C-OH), 1161cm^{-1} (C-O) and 795 cm^{-1} in FTIR spectra of H [11]. In the TiO_2 doped hydrogels, the C=O vibration intensity was decreased. Similar bending was also observed at the vibration peak of the C-O bond at 1161 cm^{-1}. In the spectra of TH, the sharp peak at 720 cm^{-1} shows the bending vibration of Ti-O-Ti. The bending vibration was seen at 1638 cm^{-1} as the short-sharp peak of Ti-OH bond.

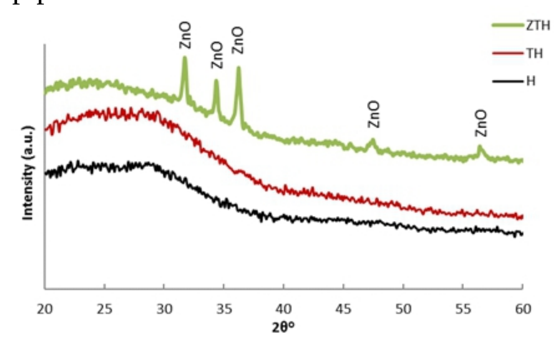

Figure 1. XRD Spectrum of H, TH and ZTH

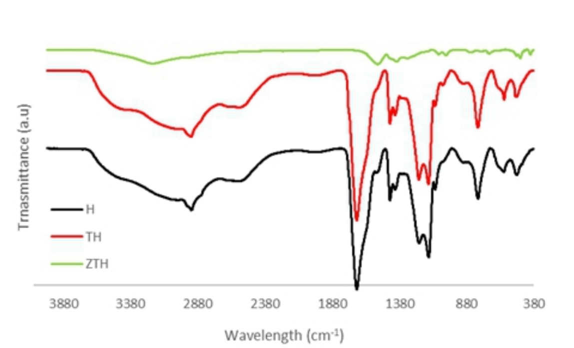

Figure 2. FTIR Spectrum of H, TH and ZTH

It should also be noted, the peak around 400–900 cm^{-1} in TiO_2 nanoparticles is related to Ti-O-Ti and Ti-O-C bond in TH sample. The additional peaks at 500 cm^{-1} could be stated to the Ti-O vibrations [10]. C-OH stretching vibrations at 1452 cm^{-1} is slightly shifted to 1415 cm^{-1} in ZTH spectra compared with H, it could indicate that the interaction between Zn^{+2} and OH. This result is in conformance with FTIR studies in literature (Dincă et al., 2018; Chen et al., 2013; Hu et al., 2011).

FESEM and EDX analyses results of H and TH were given in Figure 3 and Figure 4, respectively. While carbon content of H was 57.55 at.%, oxygen content was 42.45 at.%. When titanium was doped into the hydrogel, no significant change in oxygen and hydrogen content was observed. Titanium content of TH was also determined as 0.16 at.%.

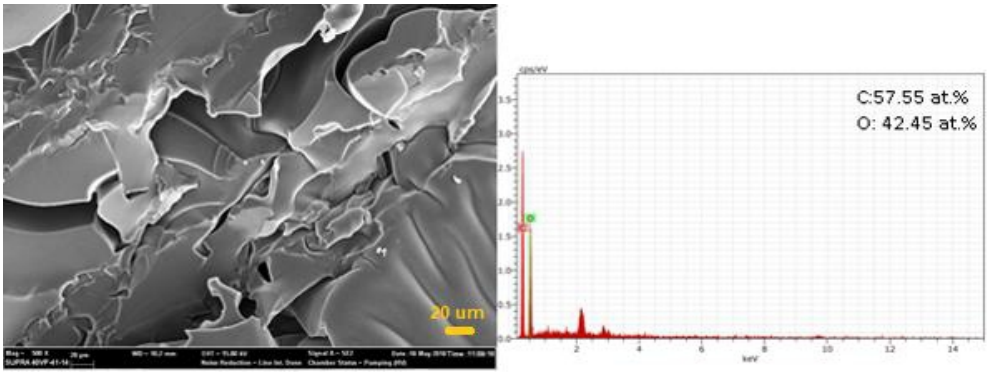

Figure 3. SEM-EDX results of H

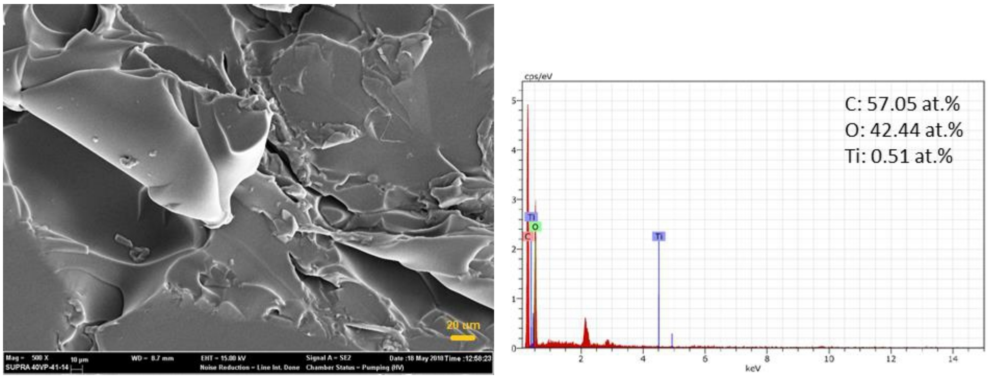

Figure 4. SEM-EDX results of TH

According to the images of nano-ZnO particles on hydrogel obtained using 15, 30, 45 and 60 minutes deposition time, the nano-ZnO structures become more apparent as the reaction time increases (Fig.5). When the deposition time of 60 minutes applied, it was observed that ZnO NPs were fully developed. EDX results of ZTH obtained at 60 min were given in Fig. 6.

Zn content of hydrogel was 35.89 at.%, Ti content of hydrogel was also determined as 0.16 at.%.

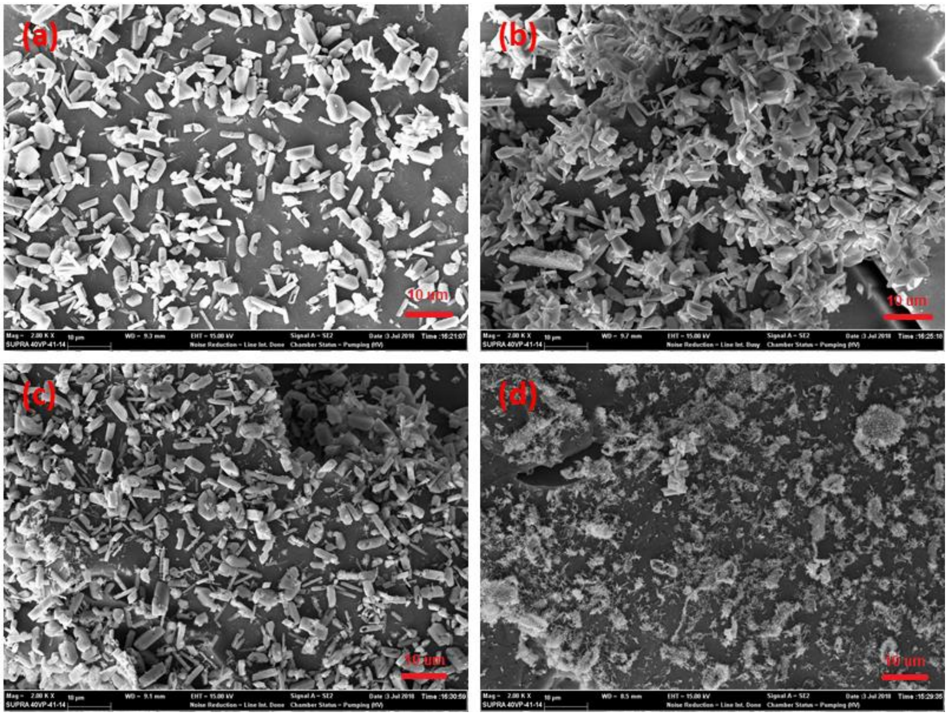

Figure 5. ZTH obtained at (a) 15 min, (b) 30 min, (c) 45 min and (d) 60 min

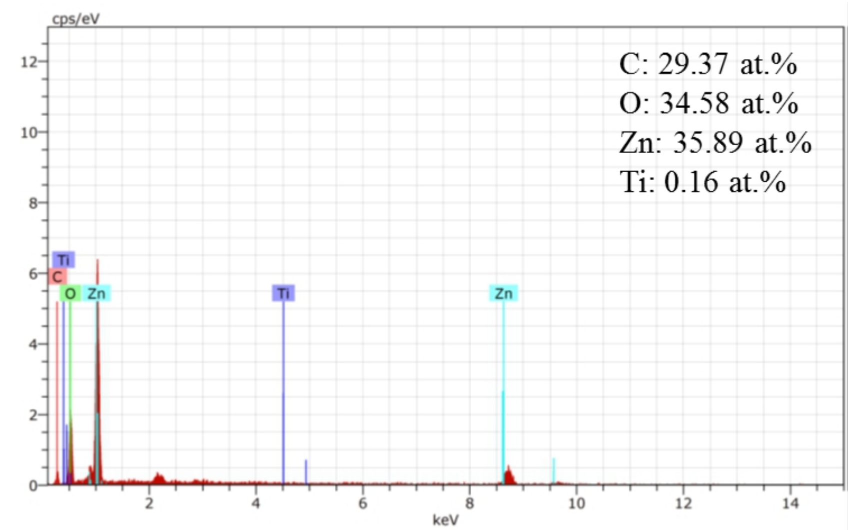

Figure 6. EDX results of ZTH obtained at 60 min

Antibacterial Activity of H, TH and ZTH

Antibacterial activity of ZnO and TiO_2 has been studied for a long while and was discussed in previous studies (Teymourinia et al., 2019;

Athanasoulia et al., 2018; Wahid et al., 2019; Jia et al., 2019). In addition to this, TiO_2-ZnO composite materials have also enhanced antibacterial property (Hwang et al., 2011). In this study, antibacterial activity tests were conducted on H, TH and ZTH with gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. The results were given in Table 1.

Bactericidal mechanism of TiO_2 and ZnO is still uncertain and is an important issue that should be investigated. Several bactericidal mechanisms have been suggested to specify the antibacterial property of TiO2 and ZnO. The mechanisms include penetration of the cell envelope by these metal oxides and damage the cell membrane with the production of reactive oxygen species (Siwińska-Stefańska et al., 2019). *Escherichia coli* were more resistant to hydrogel, TiO_2 and ZnO nanoparticles than *Staphylococcus aureus*. The enhanced property of antibacterial activity displayed by the synthesized composites, especially against *Staphylococcus aureus*, makes them promising materials for various applications.

Table 1. Antibacterial activity test results.

Sample	Bacteria	Count in the control sample after 24 hours	Count in sample after 24 hours	Decrease (%)
Hydrogel	*Staphylococcus aureus*	530000	108915	79.45
Nano-TiO_2 doped hydrogel	*Staphylococcus aureus*	530000	51064	90.37
ZnO nano-flowers deposited nano-TiO_2 doped hydrogel	*Staphylococcus aureus*	530000	129	99.98
Hydrogel	*Escherichia coli*	490000	210553	57.03
Nano-TiO_2 doped hydrogel	*Escherichia coli*	490000	94150	80.79
ZnO nano-flowers deposited nano-TiO_2 doped hydrogel	*Escherichia coli*	490000	12450	97.46

Conclusion

In this study, synthesized hydrogels were used as substrate to deposit ZnO NPs by chemical bath deposition technique. The simple synthesis method appears to be promising method as it is economical and environmental-friendly manner. The synthesized composite materials were characterized by using various analytical tools such as XRD, FTIR and FESEM-EDX. According to the characterization results, deposition time plays a key role to obtain ZnO NPs. When the deposition time of 60 minutes applied, it was observed that ZnO NPs were fully developed. Significant differences of morphological characteristics were observed with varying deposition time. Antibacterial properties of synthesized composite materials were fully determined by applying ASTME-2149 methods. The deposited TiO_2 and ZnO NPs on biodegradable hydrogel exhibited high activity against *Escherichia coli* and *Staphylococcus aureus*. Gram-negative bacteria (*Escherichia coli*) was more resistant to TiO_2 and ZnO NPs than gram-positive bacteria (*Staphylococcus aureus*). It can be concluded that, the antibacterial property of hydrogel was highly depending on doping material and the hydrogel modified by TiO_2 and ZnO have great potential for application as an inorganic antibacterial material.

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