Unravelling the Swelling Behaviour and Antibacterial Activity of Palm Cellulose Nanofiber-based Metallic Nanocomposites

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Abstract. The development of functional antimicrobial metal oxide nanocomposite systems based on nanocellulose has been the subject of extensive research in recent years. In addition to its sustainability, biodegradability and non-toxic properties, nanocelullose present an extremely high surface area favoring the selective growth and immobilization of ultrafine metal oxide nanoparticles on the cellulosic surface. In this study, oil palm biomass-derived cellulose nanofiber (CNF) decorated with zinc oxide (ZnO) nanocomposites were produced via ultrasound-assisted in situ co-precipitation approach. The morphology and chemical composition of the as-synthesized ZnO/CNF composites were characterized using field emission scanning electron microscopy (FE-SEM) and Fourier-transform infrared (FT-IR). FE-SEM images revealed the fibrous morphology of nanocomposites with a good distribution of ZnO NPs. The FT-IR analysis confirmed a strong interaction between surface functional groups of CNF and ZnO nanoparticles. The swelling behavior of composites was found to be improved with addition of ZnO nanoparticles in the CNF matrix. The hybrid ZnO-CNF exhibited pronounced antibacterial properties against methicillin-resistant Staphylococcus aureus (MRSA). The findings of present study support the possibility of using this palm CNF-based metallic nanocomposites as nanofillers for wound care application.

1. Introduction
Cellulose nanofiber (CNF) has recently attracted enormous interest due to their huge potential as green reinforcement material in polymer nanocomposites. The CNFs are commonly isolated from plants including biowaste-based sources (e.g. bamboo, cassava, pineapple, coffee waste, sorghum and eucalyptus) [1]. CNFs have shown great reinforcement effect in polymer nanocomposite due to their percolation network and large aspect ratio, besides abundant of hydroxyl groups availability that allows potential surface modification [2].
However, the susceptibility to bacterial attacks often impedes the potential industrial application of CNFs [3]. CNF loaded metal nanoparticles have been widely explored, particularly in the fabrication of wound dressing materials [4, 5]. In the field of injury care management, wound disease caused by bacteria such as Methicillin-resistant Staphylococcus aureus (MRSA) can delay the wound healing and leads to exudate development on the injured surface. Zinc oxide (ZnO) has gained considerable attention in the field of nanofiller-related research due to its high stability, good photocatalytic activity, antibacterial activity, non-toxicity and moreover it was commonly used in the manufacturing of consumer products including lotions, UV protection skin cream and skincare materials [6, 7].

Number studies have been reported on the synthesis of ZnO embedded cellulose nanocomposite. For instance, Ali and his co-workers have impregnated citrus peel isolated cellulose with ZnO, and the fabricated nanocomposite exhibit bactericidal effect towards Staphylococcus aureus (S. aureus) and Escherichia coli (E.coli) bacterial strain [8]. Most recently, cotton linter pulp cellulose have been used to fabricated cellulose/ZnO nanocomposite, that displayed antibacterial properties towards S. aureus and E. coli [9]. Although there were several reported works on antibacterial activity of CNF isolated from various sources, the potential bactericidal activity of ZnO-decorated palm CNF on MRSA and its subsequent swelling behavior, fabricated via ultrasonication assisted co-precipitation method have so far not been explored and reported elsewhere in the literature.

Herein, the goal of the present study is to investigate and antibacterial properties of ZnO-CNF nanocomposite, synthesized via ultrasonication co-precipitation technique. The ZnO-CNF were formed first by treatment with CNF in deionized water with presences of zinc acetate dihydrate (Zn(CH$_3$COO)$_2$.2H$_2$O). The zinc ions with a positive charge will absorb to the negatively charged CNF during the stirring. Addition of sodium hydroxide (NaOH) as a precursor leads to hydrolysis of zinc salt and formation of ZnO crystallite. The fabricated ZnO-CNF metallic nanocomposite was monitored for morphological characteristic, chemical component and bactericidal effect towards MRSA.

2. Materials and Methods

2.1 Materials

Palm derived CNF was kindly provided by Nanotechnology and Catalysis Research Centre, University of Malaya (Kuala Lumpur, Malaysia). Main characteristic of CNF utilized in this work include: nanofiber average diameter ~25nm, fiber content - cellulose (48%) (ASTMD 1104-56), hemicellulose (36%) (ASTMD 1103-55T) and lignin (16%) (ASTMD 1106-56). Zinc acetate dihydrate (≥ 98%), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were procured from Sigma Aldrich. Ethanol (AR standard) was acquired from R&M Chemical (Syarikat Saintifik Jaya, Malaysia). Water used in this work is ultrapure water obtained from Milli-Q® Plus apparatus (Millipore, Billerica, USA). All ultrasonication procedure was conducted using an ultrasonic horn (20kHz, 100 W system, NexTgen ultrasonic platform, Sinaptec, France) under pulse mode (15 s pulse on, 10 s pulse off). All chemicals in this study were of analytical grade.

2.2 Methods

2.2.1 Development of ZnO loaded CNF nanocomposite.

The CNF was first dispersed in water in presence of 10 mM zinc acetate dihydrate for 1 hour (h) at constant magnetic stirring speed (500 rpm). The mixture was transferred in a three-necked refluxing apparatus with Liebig condenser fixed on the middle neck and refluxed at 90°C for 1 h. The temperature of the mixture was measured using a digital thermometer (Digi Sense, 20250-01) equipped with a K-type thermocouple. Then, the mixture was treated with 100 mM concentration of NaOH to reduce zinc into nanoparticles [8]. The mixture was dispersed via magnetic stirrer at speed of 500 rpm for 30 min, accompanied by sonication for 10 min. Subsequently, the mixture was filtered and washed with ethanol and the residues were rinsed with ultra-pure water for 10 min to eliminate the excess chemical. The as-prepared wet nanocomposite was then oven-dried overnight at 40°C. On the other hand, ZnO
nanoparticles were synthesized using similar procedure as described earlier in Zn-CNf preparation but in the absence of CNf. The obtained nanoparticles were synthesized from refluxing 10 mM of zinc acetate dihydrate with 100 mM of sodium hydroxide. The mixture was refluxed at 90°C for 1 h and sonicated for 10 min. The synthesized ZnO NP were washed with ethanol and rinsed with ultra-pure water for 10 min, followed by oven drying overnight at 40°C.

2.3 Characterization
2.3.1 Morphological studies.
The surface morphology of the Zn-CNf was examined in an FE-SEM equipped with Oxford-Horiba Inca XMax50 EDX (Hitachi SU8010, Japan). All the specimens were mounted with carbon tape on aluminum stubs and then platinum coated to ensure the specimens conductive prior to FE-SEM observation. The elemental composition of the resultant Zn-CNf composite was characterized by Energy-dispersive spectroscopy (EDX).

2.3.2 FT-IR Analysis.
The chemical properties of ZnO-CNf nanocomposites were analysed using Fourier transform infrared spectroscopy (FT-IR) over a range of 550 – 4000 cm⁻¹. Each spectre was collected over 32 scans using the FT-IR spectrometer (Nicolet iS10, Thermo Scientific, USA) equipped with a diamond probe.

2.3.3 Swelling behavior.
The ZnO-CNf nanocomposites were cut into small pieces with each specimen weight of 35 mg with a diameter of ca. 5 mm. The cut piece was then immersed in phosphate buffer saline (PBS) (pH7.4) at room temperature. The nanocomposite was immersed at different time interval and thickness of the swelled sample was measured using digital electronic Vernier caliper gauge micrometer. The removed sample was gently dried with filter paper to remove excess surface water before weighing. In this study, the difference in swelling was determined by measuring the thickness of the sample according to the following equation (1)

\[
Thickness\ ratio = \frac{T_1 - T_2}{T_1} \times 100\%
\]

where \(T_1\) is the initial thickness of the sample (before immersed in solution), \(T_2\) is the thickness of the sample after immersed in the solution. The experiment was performed in three replicates.

2.3.4 Antibacterial Activity of ZnO-CNf nanocomposite.
Antibacterial activity of ZnO-CNf nanocomposite was performed using disc diffusion method. The Gram-positive bacterium (MRS13 ATCC 44300) was used to perform the antibacterial test. The method was performed using Muller Hinton medium solid agar in Petri dish. Pristine sample of ZnO-CNf was cut into a disc shape with 5 mm diameter and sterilized by UV. The disc was placed on MRS13 cultured agar plates before incubating the cultured Petri dish for 24 h at 37°C [10]. In this test, vancomycin was applied as positive control and CNf as negative control. The width (\(W\)) of the inhibition zone was determined based on equation (2) [11]:

\[
W = \frac{D_1 - D_2}{2}
\]

where \(D_1\) is the total diameter of the inhibition zone and the disc, \(D_2\) is the diameter of the disc (5mm).
3. Results and Discussions

3.1 Embedment of ZnO nanoparticles in CNF network and Morphology Characteristic

Dispersion of zinc acetate dihydrate in water induces the production of Zn$^{2+}$ ion and at the same time, CNF surfaces become slightly negative due to the ionization effect of hydroxyl group [12]. This enables Zn$^{2+}$ cation can be attached to the cellulose surface. Further, the addition of the NaOH will supply the required hydroxyl group in the suspension and results in more ionization of cellullosic chain for ZnO nucleus formation [13-15]. The proposed reaction is illustrated in figure 1 (A).

Morphological analysis of the dried CNF-ZnO nanocomposite and pristine palm derived CNF was conducted via FE-SEM and the result is shown in figure 1. Based on figure 1 (B), the ZnO particles can be clearly seen on the surface of CNF matrix with spheroid like shape agglomeration. The images implied that the use of ultrasonication promotes the uniform dispersion of ZnO nanoparticles in the cellullosic matrix having an average particle size of 28 – 34 nm. On the other hand, there are some aggregated particles size 50 – 95 nm present on the surface of CNF. Dispersed ZnO nanoparticles were indicated with arrows in figure 1 (C). On the contrary, a rather smooth surface morphology was observed for the pristine CNF specimen in figure 1 (B).

![Figure 1: Schematic illustration of the formation of ZnO-CNF nanocomposite (A), pristine CNF sample (B) and ZnO-CNF nanocomposite (C).](image)

3.2 Chemical composition and Morphology Characteristic of ZnO-CNF nanocomposite

The chemical interaction between cellulose and ZnO was affirmed using FTIR analysis and the results is presented in figure 2. The FTIR spectra of pristine CNF shows wide vibration at 3334 cm$^{-1}$ which could be attributed to the hydrogen-bonded group (OH) that shifted to 3330 cm$^{-1}$ for ZnO-CNF. The shift from lower wavenumber from 3334 cm$^{-1}$ to 3330 cm$^{-1}$ stretching vibrations of -OH groups confirmed the chemical reaction between zinc ions (Zn$^{2+}$) ions and hydroxyl groups of CNFs [10, 16]. As for pure ZnO nanoparticles, the peaks at 3377 cm$^{-1}$ is due to O-H mode of vibration while C=O shows a clear asymmetric mode of vibration at 1635 cm$^{-1}$. Similar observations have been previously reported in the case of citrus-based cellulose embedded with ZnO nanoparticles [8]. The ZnO-CNF
nanocomposites yield additional peak for ZnO vibration which was identified at 552 cm$^{-1}$. The peak was confirmed after 8 repeated runs of FTIR spectra for ZnO-CNFR to ensure the consistency of the ZnO vibration peak. The vibration peak obtained in the region between 400 and 600 cm$^{-1}$ is attributed to Zn-O bonding [17, 18].

3.3 Swelling behavior and bactericidal ability of ZnO-CNFR nanocomposite

The swelling properties were examined by measuring the differences observed in the thickness of ZnO-CNFR nanocomposite and pristine CNF composite. The measurement reported in figure 3 (A) was taken at interval of 12 hours and the thickness ratio analysis revealed that ZnO-embedded CNFs possessed slightly higher swelling capacity ca. 10% compared to the pristine CNF. The slight improvement observed in ZnO-CNFR nanocomposite swelling capacity could be possibly due to attachment of ZnO nanoparticles with various sizes, surface charges and morphology [19]. Seepage of water molecules to balance the increase of ion osmotic pressure due to presences of charged ZnO nanoparticles leads to the composite swell [19-21]. Furthermore, the presence of considerable interstitial pores in cellulosic polymeric network is deemed to have a positive influence on its liquid penetration [19, 22].

In this study, antibiotic vancomycin disc was used as positive control while negative control was pure CNF without embedment of ZnO nanoparticles. The clear zone around the disc (figure 3 (C)) was measured using the digital micrometer. The measurement was taken at least 2 different places around the disc and average value ($D_2$) was used to calculate the inhibition zone. Using equation (3), where $D_1$ is total clear zone including the disc is 9mm deducted with $D_2$ size of disc 5mm, ca. 2 mm of inhibition zone were obtained for MRSA antibacterial test, respectively. On the other hand, there was no inhibition zone were observed for CNF sample as evidenced by figure 3 (D). The antibacterial property of ZnO nanoparticles was ascribed to the generation of reactive oxygen species (ROS). Zhang et al., and Li et al., attributed the detrimental effect of ZnO to bacterial cell to the production of ROS [23, 24]. They found among ZnO produced three types of ROS, namely superoxide radicals, hydroxyl radicals and singlet oxygen. The radicals produced by the nanoparticles can result in the induction of oxidative stress, bacteria protein damage and interrupts transmembrane electron transport which will lead to DNA damage and bacterial death [25].

Figure 2: FTIR spectra of pristine CNF sample (A), ZnO-CNFR nanocomposite (B) and ZnO nanoparticles (C).
4. Conclusion

In conclusion, ZnO-CNFnano-composites were successfully produced via ultrasound-assisted co-precipitation method. The formation of ZnO on palm CNF was confirmed by FTIR analysis and morphological observation under FE-SEM. It was found that the addition of ZnO nanoparticles led to improved swelling behavior of ZnO-CNFnano-composite compared to pristine CNF. In the disk diffusion method, the tested bacteria strain was found to be present on most part of the skin and was deemed to be responsible for the observed wound contamination on the surface of the skin. The as-prepared ZnO-CNFnano-composite was efficacious in inhibiting the growth of MRSA. Our findings suggest that the developed ZnO-CNFnano-strands could be used as potential nanofillers in textures for wound dressing application.

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