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# Temperature-controlled electrospinning of EVOH nanofibre mats encapsulated with Ag, CuO, and ZnO particles for skin wound dressing

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## Abstract

To treat skin burns, a new wound dressing, nanofibre mats with metal or metal oxide nanoparticles (Ag, CuO, and ZnO), was fabricated using the electrospinning technique. During a therapeutic process, the antibacterial ability and bio-compatibility of a new dressing material are of major concern. To expound the characteristics of ethylene vinyl alcohol (EVOH) nanofibre mats encapsulated with metal or metal oxide nanoparticles, denoted as Ag-EVOH, CuO-EVOH, and ZnO-EVOH, for use as new wound dressing materials, we investigated the suitable processing parameters to fabricate these materials, such as the voltage, tip-to-collector distance, concentration of the solution, and effect of environmental temperature. The antibacterial ability and bio-compatibility of Ag-EVOH, CuO-EVOH, and ZnO-EVOH were then tested and quantified. The outcomes show that the most suitable temperature for the fabrication of the materials is 40 °C (±3 °C). The antibacterial experiment results indicate that 0.08 g/ml of metal/metallic oxide shows the highest antibacterial ability toward *Staphylococcus aureus*. Furthermore, the largest diameters of the bacteriostatic loops of the three types of nanofibre mats, i.e. Ag-EVOH, CuO-EVOH, and ZnO-EVOH, are 5.89, 5.21, and 4.12 mm, respectively. Finally, the cell proliferations on the three nanofibre mats show a similar growth trend.

Keywords: electrospinning, temperature, nanoparticles, antibacterial ability, cell proliferation

## 1. Introduction

Skin, the largest organ of the human body, is mainly responsible for protecting the tissues and organs of the body from physical, mechanical, chemical, and pathogenic invasions [1, 2]. However, in daily life, skin is subjected to more damage than that to other organs, owing to many reasons such as burns, leprosy, scabies, mycosis, etc. [3, 4]. Broadly, the most common treatment when skin is damaged is medication [5, 6]. Dressing materials are often required to assist the healing process. There are many types of wound dressings, e.g. hydrogels, films, and oil yarns. Antimicrobial dressings are currently the most widely used medical materials, and require not only antibacterial ability, but also mechanical strength, bio-compatibility, and other properties.

Many new bio-materials have been gradually introduced to replace traditional medicine for skin wound treatment, as a result of constant research efforts [7-9]. Electrospinning is a convenient and efficient method to fabricate nanofibre mats encapsulated with antibacterial substances, for use as antiseptic dressings [10,11]. However, inflammation and suppuration are still major problems that hinder treatment [12, 13].

Therefore, one of the key functions of a wound dressing material is to provide a bacteriostatic healing environment for the wound. In earlier studies, a new type of dressing material, Ag-ethylene vinyl alcohol (Ag-EVOH) nanofibre mat, with self-antibacterial ability, was fabricated using electrospinning technology [14, 15]. EVOH is a type of high-polymer resin material which consists of three elements: carbon, hydrogen, and oxygen. Moreover, the mechanical strength, modulus of elasticity, and flexural properties of EVOH are good, owing to the low flexibility of its molecular chains [16]. This material has also been approved by the US FDA for direct use on humans. The dressing material developed in this study uses nano-silver particles as antibacterial substances, to ensure its antimicrobial capacity. The antibacterial effect of silver has been proved in the subsequent antibacterial experiments [15, 17]. Furthermore, the mechanical properties of the Ag-EVOH nanofibre mats were tested for the requirements of clinical use [18].

However, the duration for the discharge of excess nano-silver particles from the human body is long, often taking months, resulting in toxicity to the human body [19, 20]. Research has shown that the toxicity of copper and zinc is less than that of silver of the same quality [21]. Hence, EVOH nanofibre mats encapsulated with nano-CuO and nano-ZnO particles are considered in this study. On one hand, copper and zinc are essential trace elements in the human body [22]. On the other, the antibacterial abilities of nano-CuO and nano-ZnO particles have been proven [23-25]. It was also found that during the fabrication process, nozzle blockages occur repeatedly. Therefore, in this study, the influence of the environmental temperature on the electrospinning process was investigated, and the suitable settings for the fabrication parameters were identified. In addition, the antibacterial properties of the three types of nanofibre mats were tested with different concentrations of metal or metal oxide supplements. Moreover, planted cell growths were observed and monitored, to determine the attachment and reproduction abilities on the fabricated mats.

This paper is arranged into the following sections: firstly, the materials and approaches used in this study are introduced, and the fabrication methods for electrospinning at different temperatures are clearly described; secondly, the micro-morphologies of the three types of nanofibre mats (Ag-EVOH, CuO-EVOH, and ZnO-EVOH), as observed by scanning electron microscopy (SEM), are presented; finally,

the antibacterial ability and the biocompatibility of the three nanofibre mats are discussed.

## 2. Materials and Methods

### 2.1 Materials

Poly(ethylene-co-vinyl alcohol) (EVOH) was used to make nanofibres; its solvent consisted of isopropanol and deionised water. The supplements, Ag particles, and CuO and ZnO nanoparticles, were supplied in the form of AgNO<sub>3</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Zn(CH<sub>3</sub>COO)<sub>2</sub>, and diethanolamine (C<sub>4</sub>H<sub>11</sub>NO<sub>2</sub>). The materials were bought from Shaanxi Huaxing Experimental Technology Co., Ltd., except EVOH, which was purchased from Sigma-Aldrich (Batch number: 12822PE), in a granular form.

The preparations of the three nanofibre mat precursor solutions were similar, in general. Isopropanol and deionised water were mixed in a 7:3 (v/v) ratio and used as the solvent to dissolve EVOH. Then, 1 g EVOH was added to 10 ml of the solvent in a conical flask, with water bath heating at 75 °C. For Ag-EVOH and CuO-EVOH, different amounts (0.2, 0.5, and 0.8 g) of AgNO<sub>3</sub> and Cu(NO<sub>3</sub>)<sub>2</sub>, respectively, were added into the flask, and stirred for 2–3 min until the solution became transparent. For ZnO-EVOH, different amounts (0.2, 0.5, and 0.8 g) of Zn(CH<sub>3</sub>COO)<sub>2</sub>, which melts at a high temperature of about 370 °C, and C<sub>4</sub>H<sub>11</sub>NO<sub>2</sub>, as the complexant, in a 1:2 (w/w) ratio, were added into EVOH solutions slowly. The conversion of the three solid substances into liquids is shown in figure 1. After stirring thoroughly, HCl was added drop-wise into the solution, till it became transparent. In total, nine precursor solutions with different concentrations of solute (0.02, 0.05, or 0.08 g/ml of Ag, CuO, or ZnO) were prepared for the process of electrospinning.

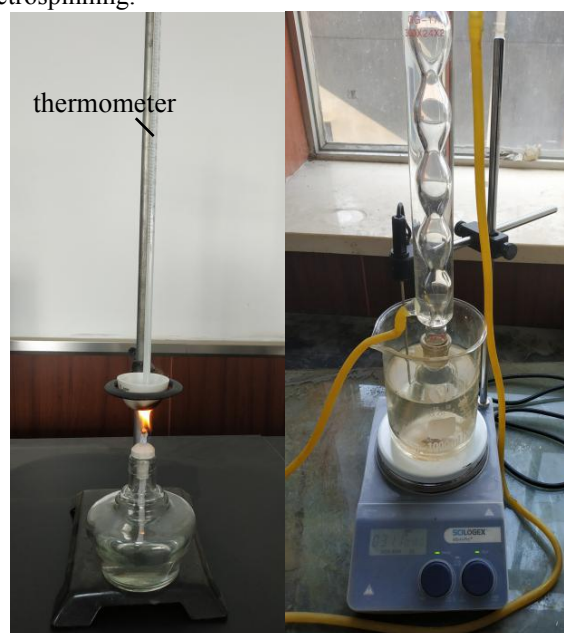


Figure 1. Preparation of three precursor solutions

## 2.2. Experimental Methods

### 2.2.1 Electrospinning Process

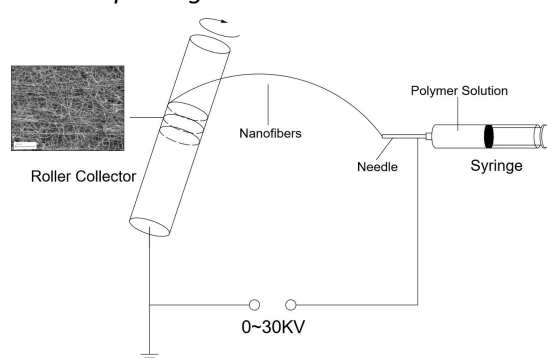


Figure 2. Electrospinning set-up

Figure 2 illustrates the fabrication setup, which consists of a syringe with a fine needle, from which a constant flow of the solution is produced into a static high-voltage electric field which provides the driving force to spin the solution jet in a spiral motion. The jet is stretched and solidified into a continuous fibre of very fine diameter, often down to tens of nanometres. The fibre can be collected in different ways, such as into a mat/sheet with or without fibre orientation control, or wound onto a roller. This process is commonly termed as electrospinning.

There are many factors that affect the final morphology of the nanofibres, such as the voltage of the static electric field, (needle) tip-to-collector stand-off distance, concentration of the solution, environmental temperature, flow speed, etc. In earlier studies, we fabricated pure EVOH nanofibre mats with 7.5–12.5% EVOH solutions, using a 20 kV voltage, 20–30 cm stand-off distance, and 0.2–0.4 mm/min flow speed, at ambient temperature (~20 °C). It was proven that the pure EVOH nanofibres were fabricated with good mechanical properties, under the aforementioned conditions [26]. However, when the same set-up was used to produce Ag-EVOH, CuO-EVOH, and ZnO-EVOH fibres, needle blockage occurred frequently. After extensive investigation through trial and error, it was found that a higher environmental temperature is needed to produce good-quality fibres. The final control parameters used for all the sample fabrications were 10% EVOH solution, 0.02 g/ml nanoparticles, 25 kV voltage, 20 cm tip-to-collector distance, and 0.2 mm/min flow speed, in a heated environment of 40 °C.

### 2.2.2 Micromorphology and Component Detection

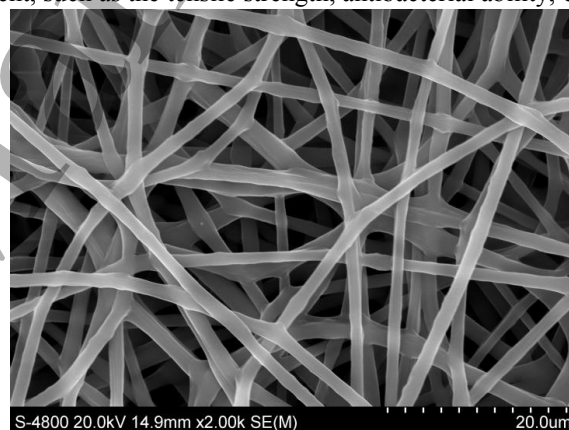
SEM and energy-dispersive X-ray spectroscopy (EDX) were used to determine the morphology and elemental

composition of the samples (figure 3). Figures 3(a) and 3(b) show the images of the pure EVOH nanofibres. The surface of the fibres appears to be fairly smooth. Furthermore, based on the EVOH spectrum, it was confirmed that there are no other elements in the EVOH nanofibres apart from the three elements C, H, and O.

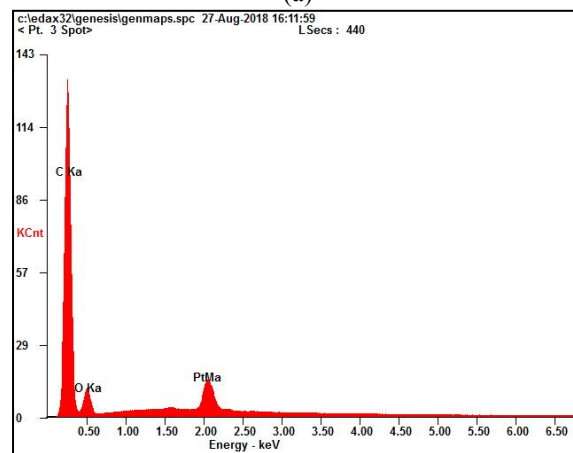
For the Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibres, a comparison with the pure EVOH nanofibres, using the images (figures 3(c), 3(d), 3(e), 3(f), 3(g), and 3(h)) and equations (1) to (3), can confirm that the small particles encapsulated in the fibres fabricated by conditional electrospinning are Ag, CuO, and ZnO nanoparticles, respectively [26–28].



Figures 3(c), 3(e), and 3(g) clearly show that the dispersity of the Ag nanoparticles is better than that of the other two. Moreover, the morphologies of the Ag-EVOH and CuO-EVOH nanofibres are more regular than that of ZnO-EVOH, although their diameters are similar. These characteristics also influenced their microscopic performance to some extent, such as the tensile strength, antibacterial ability, etc.

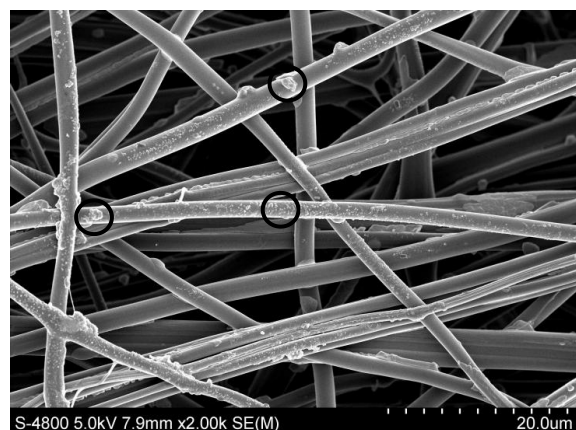


(a)

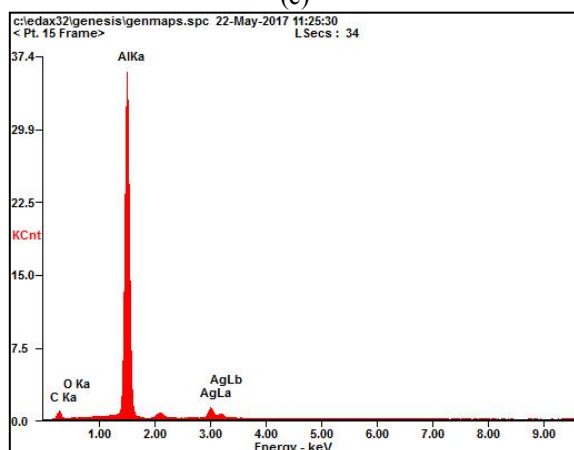


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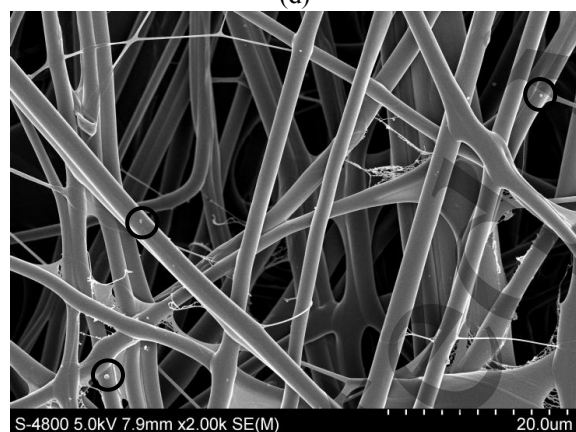




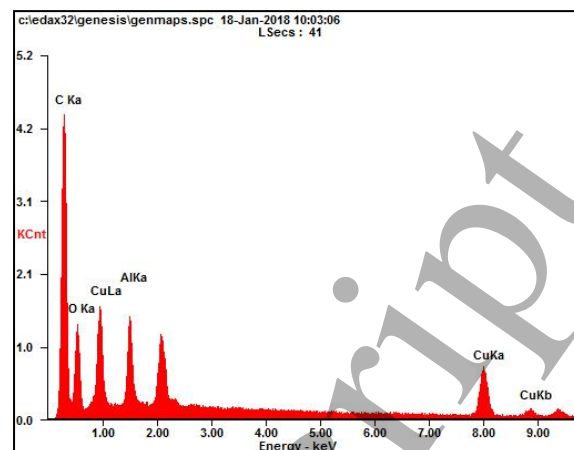
(c)



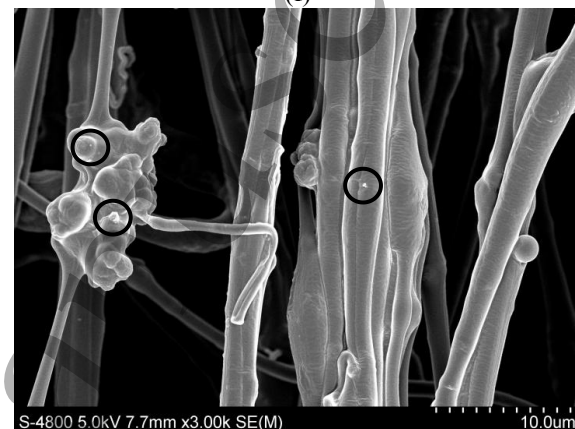
(d)



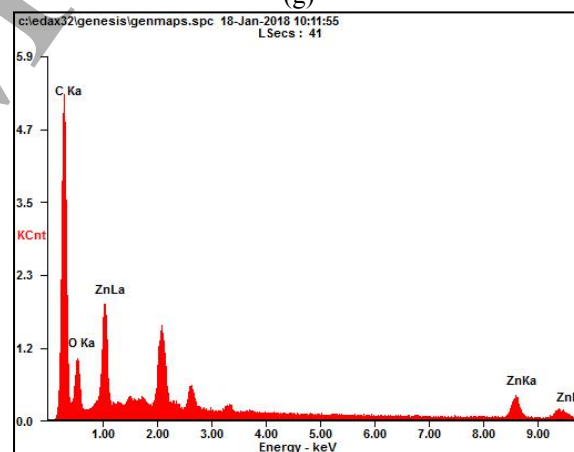
(e)



(f)



(g)



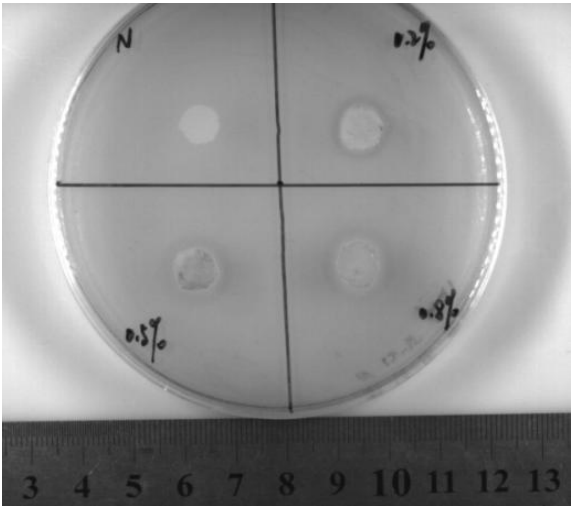
(h)

Figure 3. Morphologies ((a), (c), (e), and (g)) and energy spectra ((b), (d), (f), and (h)) of pure EVOH, Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibers (EVOH: ethylene vinyl alcohol)

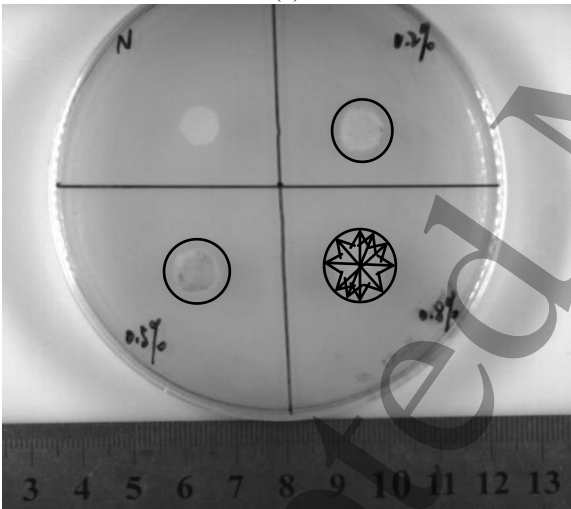
### 2.2.3 Antibacterial Test

To compare the antibacterial ability of the Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibre mats, the three samples were cut into small circular disks of diameter 8 mm.

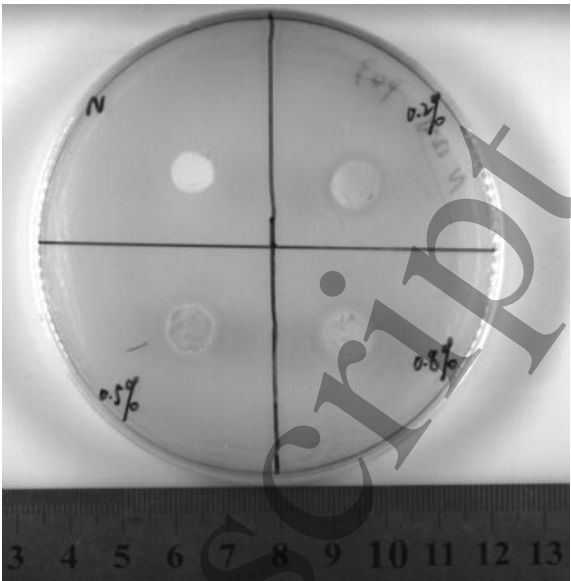
*Staphylococcus aureus*, the most common bacteria on wound surfaces, was chosen for this study. The sample mats with different concentrations of the respective nanoparticle and those of pure EVOH were placed in culture dishes coated with the bacteria. The diameter of the bacteriostatic loops formed around the sample mat disks were measured, to judge the antibacterial performance [29]. Figure 4 shows the results after 24 h of the antibacterial test. The measured diameters of the bacteriostatic loops, and their standard deviations ( $\sigma$ ), at different time points, are shown in table 1.



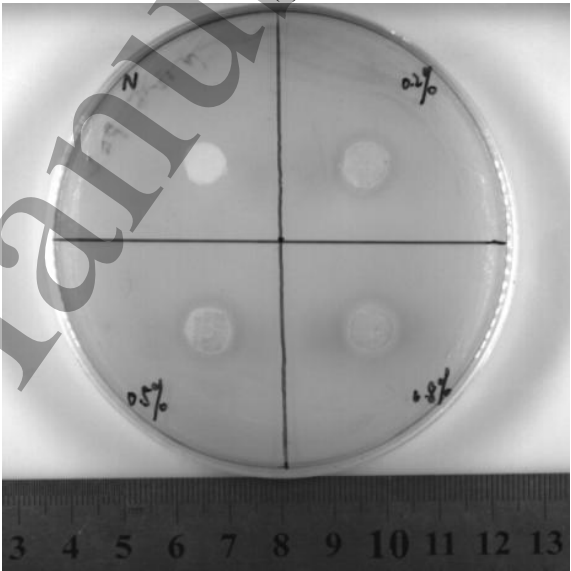
(a)



(b)



(c)



(d)

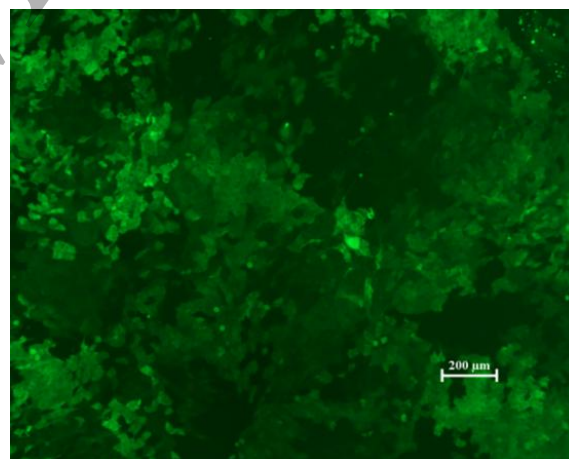
Figure 4. Antibacterial test of Ag-EVOH ((a) and (b)), CuO-EVOH (c), and ZnO-EVOH (d) nanofibre mats for 24 h (EVOH: ethylene vinyl alcohol)

Sample		Ag-EVOH	$\sigma$	CuO-EVOH	$\sigma$	ZnO-EVOH	$\sigma$
Concentration (g/ml)	Time (h)	Bacteriostatic loop diameter (mm)					
0.02	0	0	0	0	0	0	0
	6	2.65	0.19	1.67	0.21	1.95	0.12
	12	3.82	0.20	2.17	0.17	2.65	0.17
	18	3.95	0.22	2.26	0.18	2.80	0.15
	24	4.02	0.17	2.33	0.19	2.91	0.18
0.05	0	0	0	0	0	0	0
	6	3.42	0.15	2.46	0.14	2.69	0.19
	12	4.58	0.19	3.26	0.16	4.06	0.2
	18	4.86	0.16	3.49	0.11	4.38	0.17
	24	5.01	0.21	3.55	0.15	4.53	0.14
0.08	0	0	0	0	0	0	0
	6	3.86	0.17	2.95	0.17	3.16	0.18
	12	5.03	0.15	3.75	0.14	4.75	0.16
	18	5.62	0.18	4.01	0.16	5.07	0.15
	24	5.89	0.14	4.12	0.16	5.21	0.13

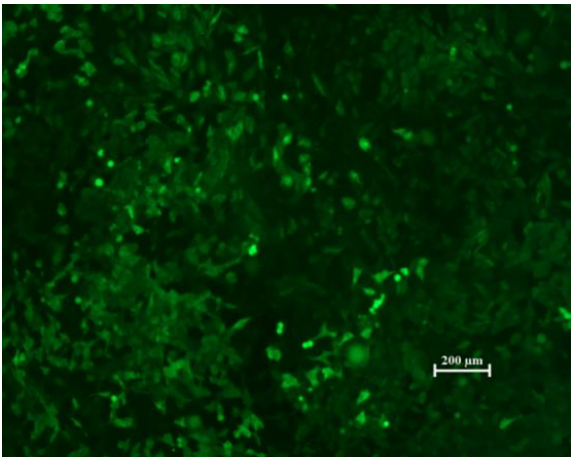
Table 1. Bacteriostatic loop diameters of Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibre mats at different time points (EVOH: ethylene vinyl alcohol)

#### 2.2.4 Cell Proliferation Experiments

To examine the capacity of the sample mats to stimulate cell growth during the healing process, cell proliferation tests were carried out on the nanofibre mats. The cells used in this experiment were Human Umbilical Vein Endothelial Cells-Green fluorescent protein (HUVEC-GFP), and the culture medium consisted of a growth medium and trypsin digest solution. The growth medium was mixed with a high-glucose medium (H-DMEM), 10% Gibco, 1% penicillin, and streptomycin sulfate. The trypsin digest solution comprised three parts, including Phosphate Buffer Saline (PBS), 0.25% trypsin, and 0.02% Ethylenediaminetetraacetic acid (EDTA). After the HUVEC-GFP cells were cultured for 2 d in the growth medium, they were inoculated into 96 orifice plates (2000 holes), onto which small pieces of the mat samples pre-treated with a hydrophile were placed. The morphology and quantity of the cells were observed using an inverted fluorescence microscope, after having been planted for 1 to 7 d at 37 °C, approximately. In addition, the absorbance (OD) at 450 nm, which is a measure of cell proliferation, was detected and assessed. Figure 5 shows the images of the proliferation of the HUVEC cells after culturing for 7 d with the pure EVOH nanofibre sample (5(a)) and 0.08 g/ml Ag-EVOH nanofibre sample (5(b)).



(a) Pure EVOH nanofibre mat



(b) EVOH nanofibre mat with 0.08 g/ml Ag  
Figure 5. Proliferation of HUVEC cells after culturing for 7 d

### 3. Discussion

#### 3.1 Influence of Temperature

When electrospinning is carried out for EVOH with metal or metal oxides, at room temperature ( $20\pm 2\text{ }^{\circ}\text{C}$ ), there is a tendency for substance attachment to the outlet of the nozzle, which hinders the formation of the Taylor cone [30]. Hence, different temperatures (30, 40, and  $50\text{ }^{\circ}\text{C}$ ) were tested, with the other parameters kept constant. We found that substance attachment was minimum at  $40\text{ }^{\circ}\text{C}$  ( $\pm 3\text{ }^{\circ}\text{C}$ ). There are two reasons for this phenomenon. Firstly, the solution in the syringe is prepared at  $75\text{ }^{\circ}\text{C}$ , and hence its temperature drops rapidly at ambient conditions, resulting in a decrease in the solubility and fluidity. Moreover, the concentration of the solute in the Taylor cone at the nozzle is high. Therefore, the solute would easily separate out at the nozzle at low temperatures. This problem was solved effectively by increasing the temperature suitably. However, when the temperature was further increased, e.g. close to  $60\text{ }^{\circ}\text{C}$ , blockages again occurred frequently. This is primarily due to the acceleration of solvent volatilisation. The solvent that we used in this study was isopropanol, which has a boiling point of  $82.45\text{ }^{\circ}\text{C}$ . Once the environmental temperature was sufficiently high, the solvent of the solution that forms the Taylor cone would volatilise before ejecting, resulting in attachment to the nozzle. Furthermore, the agglomeration of the nanoparticles increases. This is not conducive to a good overall antibacterial ability of the nanofibre mat, when it is used as a wound dressing.

Figure 6 shows the morphology of the  $0.02\text{ g/ml}$  nanofibres fabricated at  $40\text{ }^{\circ}\text{C}$ , and the measurements of the nanofibre diameter. At least 5 parts of the sample were measured for each diameter value, and the trimmed mean was taken as the average value of the sample. A comparison of the results for the three samples under different

temperatures (30, 40, and  $50\text{ }^{\circ}\text{C}$ ) is given in figure 7, showing a decreasing trend for the nanofibre diameter, in terms of the fabrication temperature.

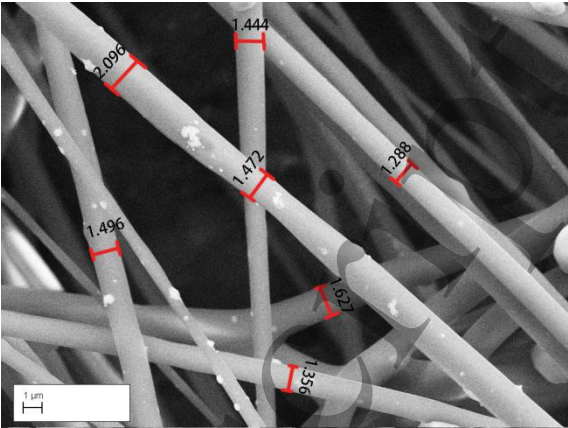


Figure 6. SEM image of  $0.02\text{ g/ml}$  nanofibres fabricated at  $40\text{ }^{\circ}\text{C}$

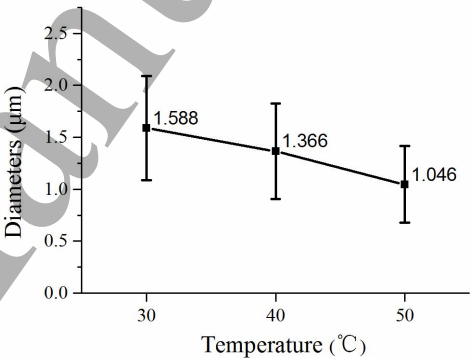
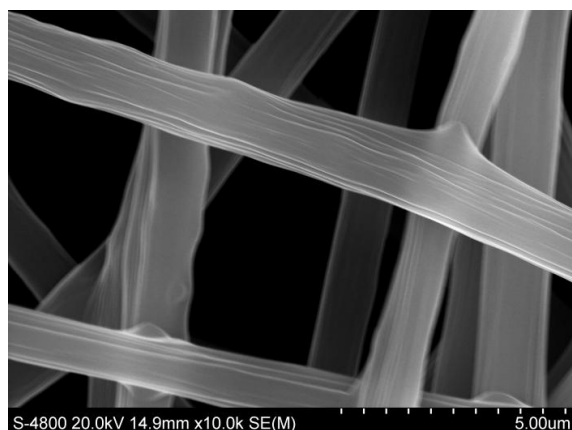


Figure 7. Influence of temperature on the diameter of nanofibres

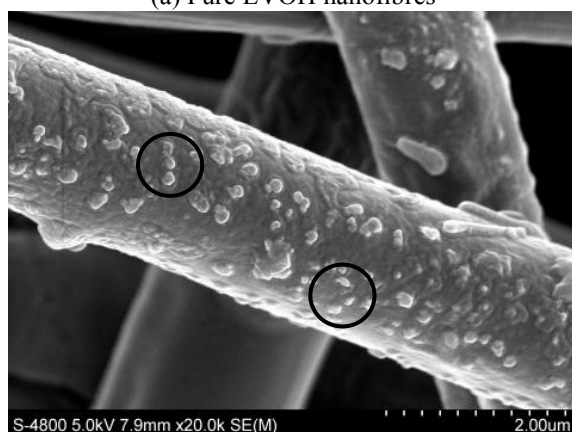
#### 3.2 Antibacterial Ability

Figures 8(b) to 8(d) show the morphologies of the Ag, CuO, and ZnO nanoparticles, respectively, attached to the nanofibre mats. Most of the particle sizes are less than  $100\text{ nm}$ , which promoted their antibacterial ability. Comparing the three images, it is clear that the Ag particles are distributed most evenly. There is a slight agglomeration of the CuO and ZnO nanoparticles. These micro-characteristics also had some effect on the antibacterial properties of the nanofibre mats.

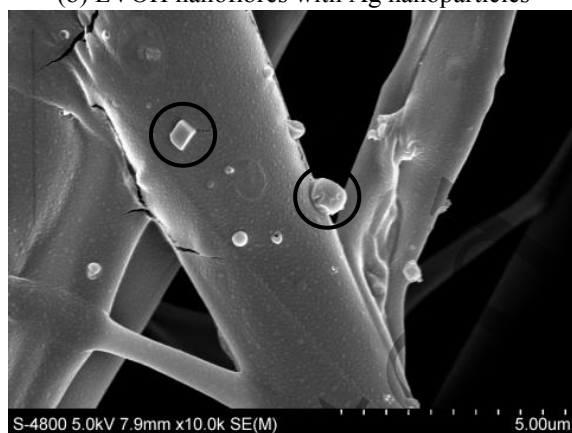




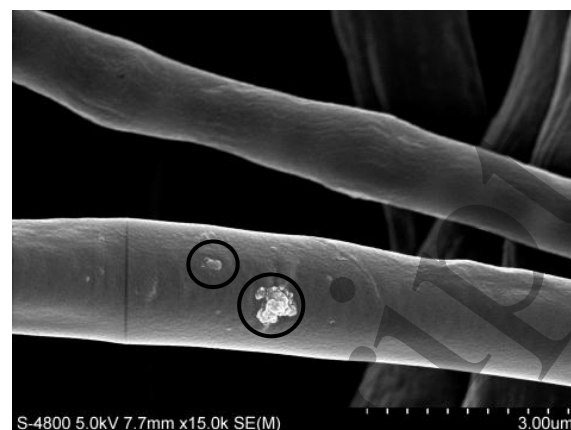
(a) Pure EVOH nanofibres



(b) EVOH nanofibres with Ag nanoparticles



(c) EVOH nanofibres with CuO nanoparticles



(d) EVOH nanofibres with ZnO nanoparticles

Figure 8. Morphology of nanoparticles in a single nanofibre (EVOH: ethylene vinyl alcohol)

Figure 9 shows the results of the antibacterial tests for the three types of nanofibre mats. Based on figures 9(a)–9(c), it can be concluded that the Ag-EVOH, CuO-EVOH, ZnO-EVOH nanofibre mats were all effective in eliminating *S. aureus*. The antibacterial ability increases with the concentration of the Ag, CuO, and ZnO nanoparticles. However, in figures 9(c)–9(f), which compare the diameters of the bacteriostatic loops of the Ag, CuO, and ZnO particles of the same concentration, the trends of the tests of the antibacterial ability the of Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibre mats are similar; furthermore, the antiseptic effect of the ZnO-EVOH nanofibre mat is weaker than that of the Ag-EVOH nanofibre mat in the first 6 h, but then exhibits a similar effect. The main reason for this could have been the way ZnO was added to the EVOH solution. It is more difficult to produce ZnO from  $\text{Zn}(\text{CH}_3\text{COO})_2$  than producing Ag and CuO from  $\text{AgNO}_3$  and  $\text{Cu}(\text{NO}_3)_2$ . The ZnO-EVOH precursor solution was mixed by fully melting zinc acetate and dissolving it in the EVOH solution, rather than adding  $\text{Zn}(\text{CH}_3\text{COO})_2$  to the EVOH solution directly, as in the preparation of the Ag-EVOH and CuO-EVOH solutions. Therefore, the dispersion of the ZnO nanoparticles in solution is different from that of the Ag and CuO nanoparticles. Figures 3 and 8 also show that the total surface area of the ZnO nanoparticles is lower than that of the Ag and CuO nanoparticles, thus requiring a longer release time. This could be the reason why it worked slowly [31].

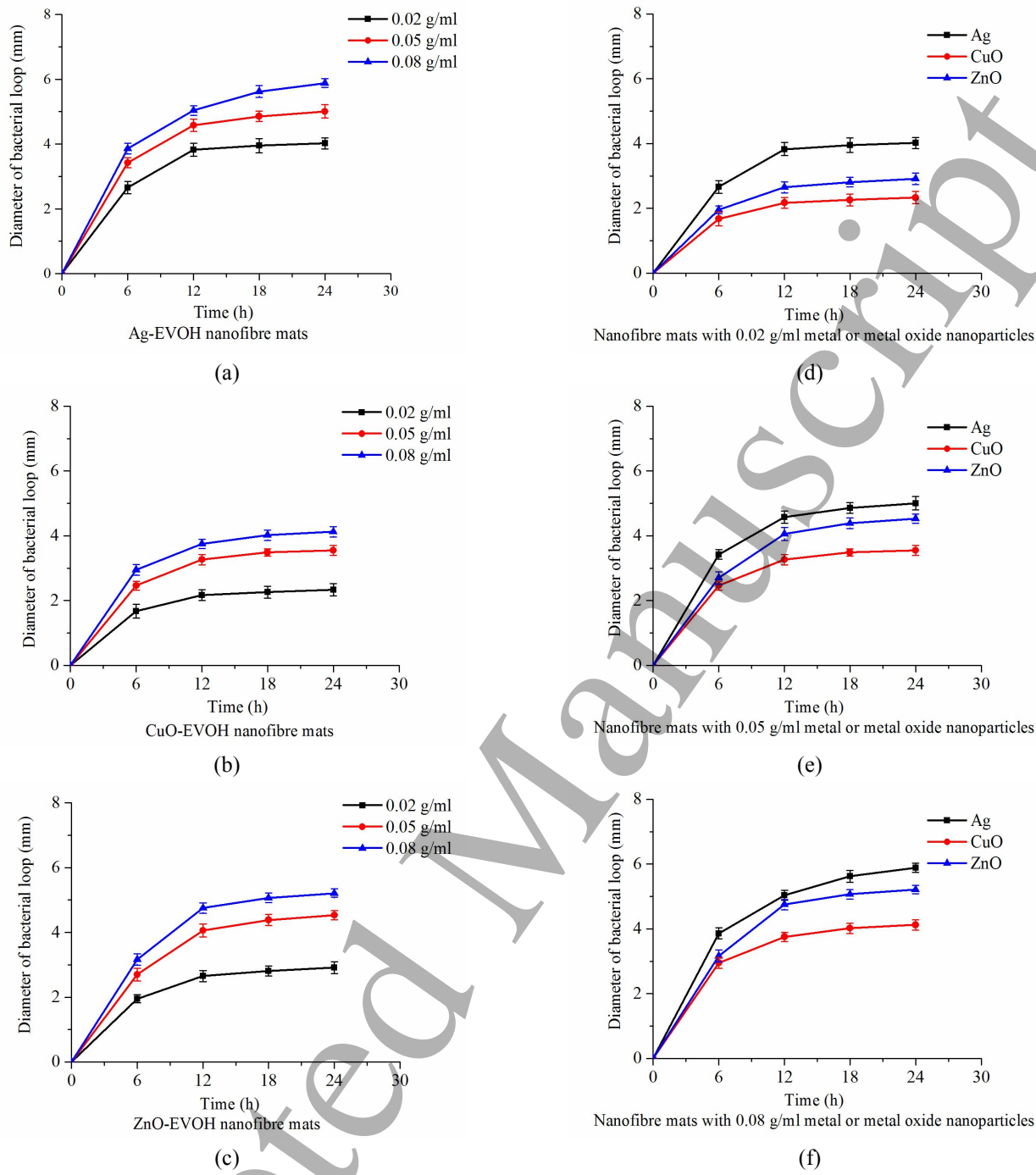
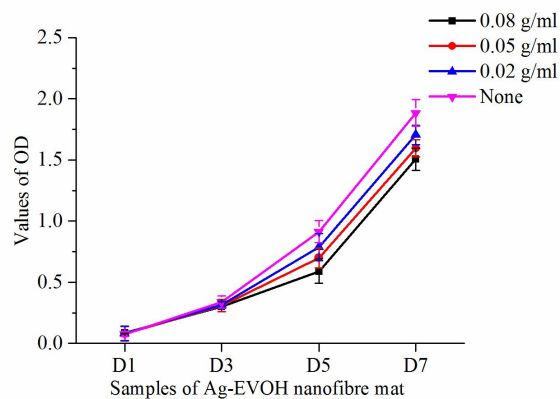


Figure 9. Results of the antibacterial test for the three types of nanofibre mats

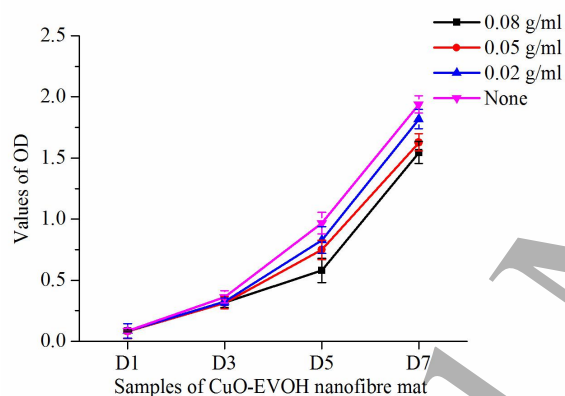
### 3.3 Cell Proliferation

Figure 10 shows the values of absorbance (OD) for the different samples. It indicates that after inoculation for 24 h, the HUVEC cells can be attached to the EVOH film in shuttle form. The HUVEC cells proliferated on the Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibre mats in a similar manner. However, the growth of the HUVEC cells on

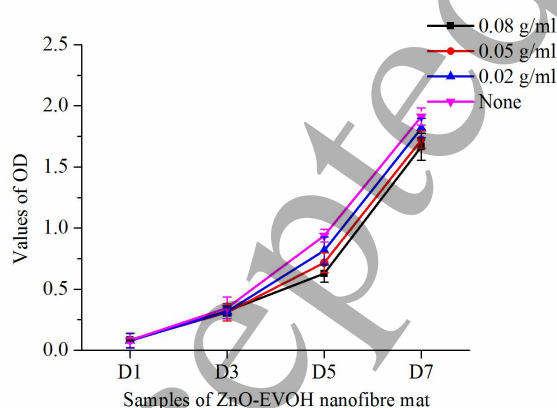
the nanofibre mats with nanoparticles was slightly lower than that on the pure EVOH nanofibre mats. This can also be regarded as clear evidence of the biocompatibility of EVOH nanofibre mats, which have been studied previously [32]. It is notable that the metals and metal oxides cannot be the bases for cell proliferation, and their presence seems to have some, although apparently limited, effect on the inhibition of cell growth.



(a)



(b)



(c)

Figure 10. Values of OD for different samples

#### 4. Conclusions

In this study, Ag-EVOH, CuO-EVOH, and ZnO-EVOH nanofibre mats were fabricated by electrospinning. Experimental research was carried out to verify the effect of temperature, and the biological application performance of the mats obtained. This led to the following conclusions.

- (1) Environmental temperature has a significant effect on the electrospinning process, mainly due to the phenomenon of needle blockage. The experimental investigations led to the conclusion that the most suitable temperature for electrospinning of EVOH nanofibre mats encapsulated with Ag, CuO, and ZnO particles is about 40 °C.
- (2) In antibacterial tests using *S. aureus*, the Ag-EVOH nanofibre mats showed the maximum antibacterial capacity, whereas the CuO-EVOH nanofibre mats were the weakest. The antibacterial mechanisms of Ag, CuO, and ZnO are complex [33-35]. These metal or metal oxide particles continuously release metal ions into aqueous media; these ions can penetrate the cell walls of bacteria and destroy the activity of cell synthetase, disrupting their division and proliferation. Metal ions are then freed from the cells after sterilisation, and continue to kill other bacterial cells. This is the mechanism for the persistent antibiotic activity of metals or metal oxides.
- (3) Cell proliferation experiments showed that the HUVEC cells proliferate with a similar growth trend for all the samples. However, the presence of metal or metal oxide particles on the surface of the fibres may hinder cell growth to a small extent, compared with that on pure EVOH fibre mats.

This study shows that EVOH nanofibre mats encapsulated with metal or metal oxide (Ag, CuO, and ZnO) nanoparticles, prepared by electrospinning, demonstrated antibacterial and cell growth capacities. With studies on other characteristics such as the mechanical and biodegradable properties, they could potentially be used as wound dressing materials, for improved functionality in the wound healing process. We intend to carry out further studies and more tests on a broader range of samples and conditions.

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