Journal of Engineering Science and Technology
5th EURECA 2015 Special Issue March (2017) 68 - 81

PREPARATION AND CHARACTERIZATION OF CU-, FE-, AG-, ZN- AND NI- DOPED GELATIN NANOFIBERS FOR POSSIBLE APPLICATIONS IN ANTIBACTERIAL NANOMEDICINE

T. NUGE[1], K. Y. TSHAI[1,*], S. S. LIM[1], N. NORDIN[2], M. E. HOQUE[1]

[1]University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia
[2]Faculty of Medicine and Health Science, University Putra Malaysia,
Lorong Asam Jawa 1, 43400 Serdang, Selangor, Malaysia
*Corresponding Author: kim-yeow.tshai@nottingham.edu.my

Abstract

A facile and efficient approach to prepare metal nanoparticles doped electrospun gelatin from metal salts precursors was successfully developed. The incorporation of metal ions with antimicrobial activity into electrospun gelatin (Ge-espun) is an attractive approach to control the inflammatory reaction and prevent infection in wound. In this study, metal salts precursors $AgNO_3$, $Fe(NO_3)_3.9H_2O$, $Cu(NO_3)_2.3H_2O$, $Zn(NO_3)_2.6H_2O$ and $Ni(NO_3)_2.6H_2O$ were reduced to metal nanoparticles with acetic acid as solvent and reducing agent. The agglomeration of nanoparticles was inhibited by the gelatin polymer matrix. Electrospinning of both neat Ge-espun and metal-nanoparticles/Ge-espun resulted in the formation of smooth fibres with average diameters of ~280nm and ~40nm-150nm, respectively. The efficacy of metal nanoparticles/Ge-espun against bacteria commonly found on wounds was tested with different metal loading by measuring the inhibition of colony forming units. The results indicated a broad spectrum of antibacterial activity showed by Ag/Ge-espun, followed by Fe/Ge-espun and Zn/Ge-espun. An interesting finding on the efficacy of Cu/Ge-espun and Fe/Ge-espun against the Gram's positive bacteria is worth exploring to further investigate the potential application of metal-based antibiotics against the antibiotic-resistant bacterial strains.

Keywords: Electrospinning, Antibacterial, Metal-nanoparticles, Electrospun.

1. Introduction

In recent years, electrospinning has gained much attention as a useful method to prepare fibres in nanometre diameter range. The unique combination of high

Nomenclatures

Ag	Silver
Ag^{+}	Silver(I) cation
$AgNO_3$	Silver nitrate
Cu	Copper
Cu^{2+}	Copper (II) cation
$Cu(NO_3)_2$	Copper(II) nitrate
$Cu(NO_3)_2.3H_2O$	Copper(II) nitrate trihydrate
Fe	Iron
Fe^{3+}	Ferric cation
$Fe(NO_3)_3.9H_2O$	Iron(III) nitrate nonahydrate
M^{n+}	Metal(n) cation
M_0^n	Metal nanoparticle nuclei
ne^-	Amino, carboxyl or hydroxyl anion
Ni	Nickel
Ni^{2+}	Nickel(II) cation
$Ni(NO_3)_2.6H_2O$	Nickel(II) nitrate hexahydrate
Zn	Zinc
Zn^{2+}	Zinc (II) cation
$Zn(NO_3)_2.6H_2O$	Zinc nitrate hexahydrate

Abbreviations

CFU	Colony-forming units
ddH_2O	Deionized distilled water
EDX	Energy-dispersive X-ray
Espun	Electrospun
Ge-espun	Electrospun gelatin
SEM	Scanning electron microscopy
UV-Vis	Ultraviolet–visible spectroscopy

specific surface area, extremely small pore size, flexibility and superior mechanical performance makes electrospun (espun) nanofiber a preferred material for many applications especially in biomedical fields such as enzymes immobilization, tissue engineered scaffolds and drugs deliveries [1].

In an electrospinning process, a polymer solution held by its surface tension and a high voltage is applied to the liquid solution at the end of a capillary tube. Charge is induced on the liquid surface by an electric field. Mutual charge repulsion causes a force directly opposite to the surface tension. As the intensity of the electric field is increased, the hemispherical surface of the solution at the tip of the capillary tube elongates to form conical shape known as the Taylor cone [2]. When the electrical field reaches a critical value at which the repulsive electric force overcomes the surface tension force, a charge jet of the solution is ejected from the tip of the cone [3]. Since this jet is charged, its trajectory can be controlled by an electric field. As the jet travels in the air, the solvent evaporates, leaving behind a charged polymer fibre which lays itself randomly on a collecting metal plate. Thus, continuous fibres are laid to form nonwoven fabric.

Overall, this is a relatively robust and simple technique to produce nanofibers from a wide variety of polymers including gelatin. Gelatin is a natural biopolymer derived either by partial acid (gelatin type A) or alkaline hydrolysis (gelatin type B) of animal collagen from skins, bones and tendons [4]. The differences in collagen sources and preparation techniques make gelatin present a structure with variable physical properties and chemical heterogeneity [5]. Due to its natural abundance and inherent biodegradability in physiological environments, gelatin is widely used in food, cosmetic, pharmaceuticals and medical applications [6]. Gelatin can be processed into hydrogels, three dimensional microporous scaffolds and electrospun into non-woven nanofibrous matrices due to its inherent properties such as triple helix conformational structure [6]. A concise review on the latest research advancement in gelatin-based electrospun nanofibres membrane could be found in the authors' earlier publication [7]. The electrospun gelatin (Ge-espun) nanofibers are ideal materials to be used as scaffolds for cell and tissue culture, carriers for tropical/transdermal delivery of drugs and wound dressing. Owing to its remarkable properties, such as high surface area to volume ratio, high porosity of the electrospun matrices and flexibility for surface functionalization (via pendant free amine groups), the electrospun gelatin matrices are of interest in the current study.

The high water absorption capacity and ability to activate macrophages and homeostasis in bleeding wounds are highly desired properties for wound dressing [8]. More recent strategies on controlling the inflammatory reaction and preventing infection in wounds are achieved via the incorporation of metal-based antibiotics into nanofibrous matrix [9]. Wound infection delays wound closure, diminishes tensile strength of the healing wound tissue, increases the length of hospital stay and cost, and increases the patient's risk of bacteremia, sepsis, multisystem organ failure and death [10]. With the increased prevalence and the emergence of antibiotic-resistant bacterial strains, there is mounting need to reduce and eliminate wound infection using methodologies that limit the ability of bacteria to evolve into further antibiotic resistant strains.

Klinkajon and Supaphol [11] investigated the use of copper (II) ions in hydrogel alginate dressing. The results showed that the copper (II) ions exhibited bacterial disinfection and displayed potentiating effect on prothrombotic coagulation and platelet activation. Additional work by Chaturvedi and coworkers demonstrated that the copper ions were successfully impregnated onto polyvinyl alcohol (PVA) based cryogels nanocomposites and exhibited high antibacterial activity against Gram-positive and Gram negative bacteria. The nanocomposites also offered fair blood compatibility and good mechanical strength [12]. A study by Lin et al. [13] aimed to investigate the efficacy of silver containing dressings on controlling wound infection and wound healing. Silver ions and silver based compounds are known to have broad antibacterial spectrum (highly toxic to 16 major species of bacteria) [14] and are relatively safe, which makes silver an excellent choice for clinical care and consumer products. The impregnation of silver ions onto scaffolds could enhance reduction in the levels of pro-inflammatory cytokines [15] and inhibit the activities of interferon gamma and tumor necrosis factor alpha which are involved in inflammation [16]. Recent investigations have been extended to study other metal nanoparticles such as Zn, Ni and Fe that could have antibacterial activity and are economically cheaper than Ag and Cu.

This works describes the preparation and characterization of Ge-espun chelates with metal ions (Cu^{2+}, Zn^{2+}, Ag^{+}, Ni^{2+} and Fe^{3+}) by electrospinning technique to explore the role of metal-based antibiotics as potential antibacterial agents. In the present study, the chelation of metal ions in the gelatin solution was extensively investigated, with the aim of understanding the effects of aging time on chelation. The nanofibers features produced during the electrospinning were studied and the efficacy of the doping process onto the nanofibers was characterized systematically. In order to evaluate the antibacterial activity, the espun nanofibers doped with metal ions were subjected to antibacterial screening against three bacterial strains that are commonly found on burn wounds, of which two were Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and one Gram positive (*Staphylococcus aureus*).

2. Experimental Details

2.1. Materials

Gelatin powder and silver nitrate ($AgNO_3$) were purchased from Merck (Germany). Iron nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$), copper nitrate trihydrate ($Cu(NO_3)_2.3H_2O$) and nickel nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$) were purchased from Systerm®. Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) was purchased from R&M chemicals (UK). The solvent used was analytical grade glacial acetic acid obtained from Fisher Chemical (UK) and deionized distilled water (ddH_2O) which was filtered by Milli-Q integral water purification using 0.22µm Millipak membrane filter. All chemicals were of analytical grade and used without further purification.

2.2. Preparation and characterization of gelatin solutions containing metal ions

The metal ions chemical reagents were first dissolved in a quantity of 10% (w/v) in 90:10 glacial acetic acid/distilled water, respectively. 1% (v/v) of the metal ion mixtures were then transferred into the 18% (w/v) gelatin solution of acetic acid/ddH_2O at the ratio of 90:10. The mixture was then stirred to homogenize and allow the chelation of metal ions into the gelatin/acetic acid/water solution. In this system, gelatin was used as a stabilizer and dispensing agent for the metal ions. The gelatin solution containing metals ion were stirred for a varied period in order to investigate the effect of aging time on the formation of metal ions. The diffusion of metal ions into the gelatin/acetic acid/water solution was confirmed by monitoring the surface plasmon absorption band using UV-Vis Lambda 35 spectrophotometer (Perkin Elmer, USA).

2.3. Preparation of neat and doped electrospun gelatin matrices

The neat gelatin solution and the metals ion-containing gelatin solution that had been aged for 12 hours were fabricated into nanofibers matrices by electrospinning. The experimental set-up used for electrospinning process (Fig.1) consisted of a syringe and a needle, a ground electrode (aluminum collector plate of 20cm × 20cm wrapped with aluminum sheet) with a distance of 10cm from the

needle and a high voltage adjustable DC power supply (Gamma High Voltage Research, USA).

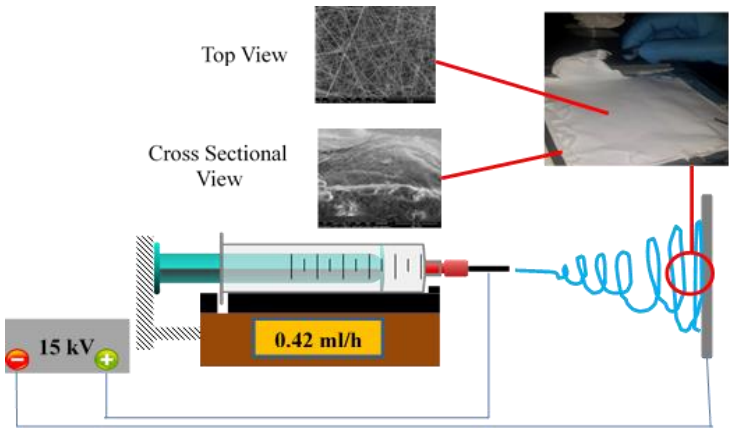

Fig. 1. Schematic of electrospinning system.

A positive voltage (15kV) was applied to the gelatin solutions. The solutions were delivered via syringe pump to control the mass flow rate. The feed rate was maintained at 0.42mL/h throughout the study. As the electrical potential was applied, a jet was created. The resulting nanofibrous matrices were collected on an aluminum plate after 18 hours of electrospinning process. After the process, all the matrices collected with the aluminum foils on the collector were kept at room temperature for 24 hours before being place in a vacuum drying oven for a couple of days for drying treatment. After the aluminum foil was carefully peeled off, the specimens' thickness was measured using a digital micrometer having a precision of 1μm. The thickness of the neat and the metals-ion containing gelatin matrices were between 320μm to 340μm.

2.4. Characterization

The nanofibers morphology was observed under scanning electron microscopy (SEM) using a FEI Quanta 400 F (USA) with acceleration voltage of 20 kV. Before SEM observation, a small section of the nanofiber mat was cut at three different positions and placed on the SEM sample holder. The diameter of at least 100 different positions on the nanofiber mat was analyzed using image visualization software ImageJ developed by Upper Austria University of Applied Sciences and available for free at http://imagej.nih.gov/ij/download.html.

2.5. Antibacterial evaluation

The antibacterial activity of the Ge-espun with both the neat and loaded metal nanoparticles were tested against bacteria commonly found on wounds: *Escherichia coli* (Gram negative: ATCC 25922), *Pseudomonas aeruginosa* (Gram negative: ATCC 27853); and *Staphylococcus aureus* (Gram Positive: ATCC 25023). The assessment was conducted based on the discs' diffusion

method of the US Clinical and Laboratory Standard Institute (CLSI). Both the neat and metal loaded gelatin matrices were cut into circular disks (7 mm in diameter). The bacteria were cultured on Luria Bertani agar (Merck, Germany) in a plastic petri dish. Initial bacteria concentration of 4.67×10^5, 5.75×10^5 and 1.95×10^5 colony-forming units (CFU) of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, were used to inoculate the culture. The nanofibrous disks were placed in the middle of culture and then incubated at 37ºC for 24 hours. If inhibitory concentration was reached, there would be no bacteria growth and could be observed as a clear zone around the disc specimens. These were photographed for further evaluation.

3. Results and Discussion

3.1. Formation of metal nanoparticles in gelatin solutions and effect of aging time

The liquid phase chemical reduction technique discovered by Faraday more than 150 years ago has been widely used for the production of metal nanoparticles. The chemical reduction method is based on mixing a reducing agent with a metal salt precursor in the presence of stabilizing agents (ligands, polymers and surfactant). The stabilizing agent is confined to prevent the undesirable particle agglomeration and the formation of stable nanoparticles depends on the properties of stabilizing agent [17]. In this study, the metal salts were reduced to metal nanoparticles with acetic acid as a solvent and reducing agent. The gelatin polymer matrix was used as stabilizer to prevent further growth and particle agglomeration of metals. The metal ions were reduced directly into metal nanoparticles through a series of steps including nuclei formation, crystal growth via diffusion mechanism to give primary particles and spontaneous self-organization of primary particles to form clusters. The formation of metal nanoparticle nuclei was postulated to originate from ionic interactions between metal ions and amino, carboxyl and hydroxyl groups on gelatin. The metal nanoparticles–gelatin matrix clusters can be expressed according to Eq. (1).

$$x\mathrm{M}^{n+} + xne^{-} + \text{Gelatin} \rightarrow \mathrm{M}_0^{\mathrm{n}} \text{ (Cluster)} \quad (1)$$

The reduction of metal ions into metal nanoparticles in metal salts-containing gelatin solutions at different aging time could be visualized from the solution color intensity, from light color of the neat solution to dark color after the completion of aging. The UV/visible absorption spectra of metal salt-containing gelatin solution that had been aged for various time intervals after preparation are displayed in Fig. 2. The surface plasmon absorption bands for $Cu(NO_3)_2$–containing gelatin solutions exhibit two peaks in the range of 370nm to 390nm and 690nm to 710nm (Fig. 2A). Upadhyaya et al. [18] observed the same pattern described that the spectral shift was due to the complexation between Cu^{2+} ions and stabilizing agents (polyaniline) and depended on the concentration of Cu^{2+} ions. Evidently, an increase in the aging time resulted in the observed color change in solution from blue to yellowish green and finally to dark green. It can be assumed that the cupric ions were bound to gelatin chains. Similar redox behavior was observed with Fe^{3+}, Ni^{2+} and Zn^{2+} ions in this study.

The reduction of Ag^{+} to Ag^{0} (silver nanoparticles) by acetic acids and the formation of silver nanoparticles-gelatin matrix follows the following schemes, Eq. (2) and Eq. (3) [19]:

$$H_2N{-}COOH + AgNO_3 \leftrightarrow H_2N{-}COOAg + HNO_3 \quad (2)$$

$$H_2N{-}COOH + AgNO_3 \leftrightarrow AgHN{-}COOH + HNO_3 \quad (3)$$

Silver nanoparticles have a surface plasmon resonance absorption in the UV-visible region and the band arises from the coherent existence of free electrons in the conduction band due to small particle size [20]. The band shift is dependent on the particle size, chemical surrounding, adsorbed species on the surface and dielectric constant [19]. Fig. 2B shows the UV-Vis spectra gradually increased over time and the peak position was slightly shifted to a longer wavelength. This indicates that the number of Ag^{+} ions that converted into silver nanoparticles increased with an increase of reaction time. The absorption peaks were observed at 382nm and 420nm, which correspond to two particle population of silver nanoparticles.

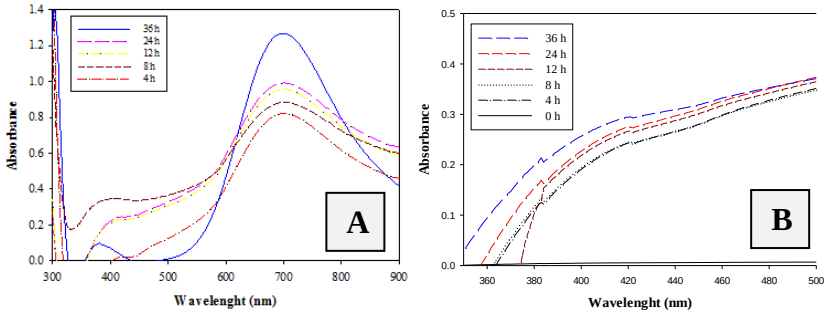

Fig. 2. Surface plasmon absorption band of aged (A) $Cu(NO_3)_2$ and (B) $AgNO_3$ containing gelatin solution using UV-Vis spectrophotometer at different time intervals. The concentration of gelatin solution was 18% (w/v) and the metal salt precursor was 1 % (w/w) based on the weight of gelatin.

3.2. Morphology of metal nanoparticles / Ge-espun nanofibers

While electrospinning has proven to be a versatile and powerful mean for fabricating nanofibers, the applicability to obtain smooth, uniform fibrous structure is not straightforward. The morphology and diameter of espun fibres are dependent on a number of parameters including properties and composition of the polymer solution such as viscosity, concentration, conductivity and surface tension [21]. The optimum parameters for fabricating smooth and uniform neat espun gelatin nanofibers were discussed in our previous work [22].

Fig. 3 shows the selected SEM images of the samples generated using electrospinning from gelatin solution and gelatin solutions containing different metal salts as a precursor. The conductivities of the metal nanoparticles-gelatin solutions linearly increased, while the viscosities and surface tensions did not change with increase content of metal salts. Since the metal salts increased the charge density in gelatin solutions, stronger stretch forces were imposed on the ejected jets under the electrical field, resulting in substantial formation of finer

ultrafine fibres [23]. The average diameter of neat Ge-espun and Ge-espun loaded with metal nanoparticles are shown in Fig. 3. Smooth and uniform nanofibers with diameter in the range of ~ 200 to 360 nm and average diameter of 280.60 ± 35.92 nm were observed on the neat electrospun gelatin (Fig. 3A). The average diameters tended to decrease with nanometals loaded fibres. The average diameters of Ag/Ge-espun and Cu/Ge-espun were 147.33 ± 96.43 nm and 41.43 ± 8.05 nm, respectively (Fig. 3B and 3C). The average diameters of Fe/Ge-espun, Ni/Ge-espun and Zn/Ge-espun were ranging from 45.21 ± 12.43 nm to 102.05 ± 26.12 nm (data not shown). Wide variation of nanofiber size was observed on the Ag/Ge-spun. The diameter of Ag/Ge-spun obtained was much smaller compared to the study reported by Rujitanaroj et al. [24] with the diameter ranging from 280 ± 40 nm.

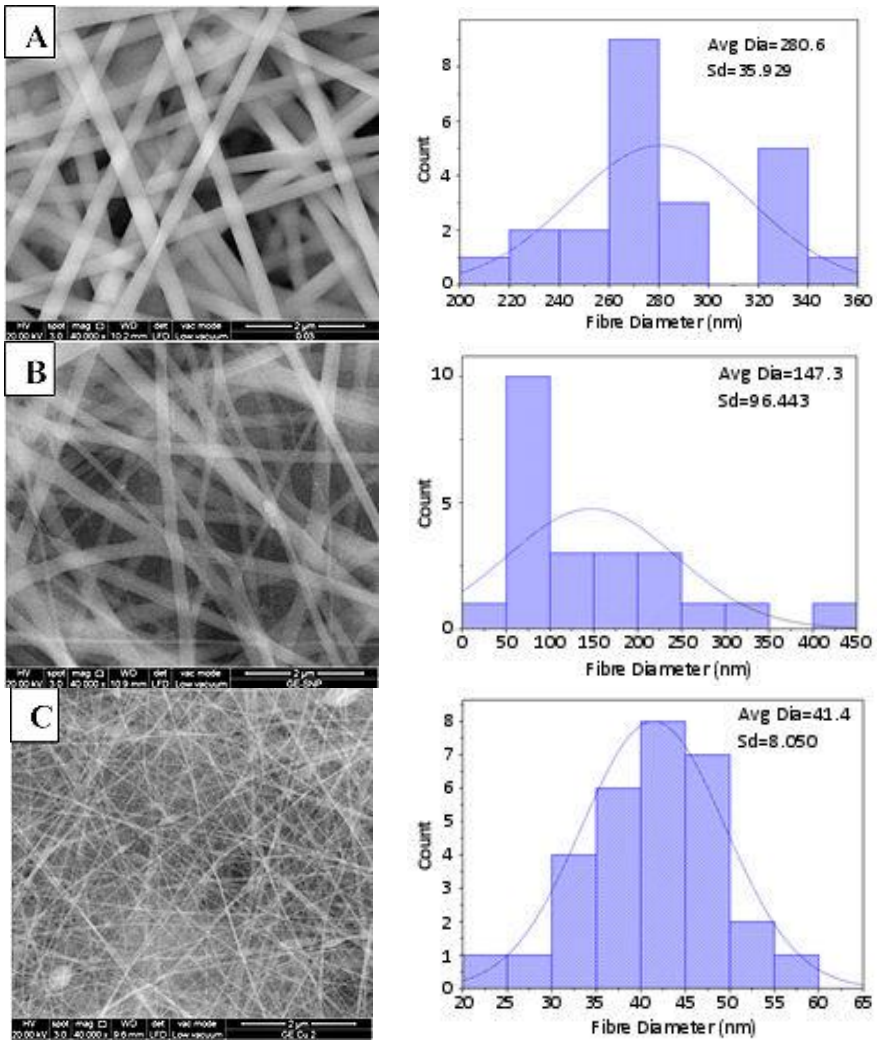

Fig. 3. SEM micrograph of the Ge-espun nanofibers and diameter distribution of (A) neat Ge-espun (B) Ag/Ge-espun (C) Cu/Ge-espun

The presence of metal nanoparticles in the nanofibers was further assessed with energy-dispersive X-ray (EDX) spectroscopy analysis. The assessment proved the presence of metal nanoparticles in espun. The quantitative analysis of the presence of metal nanoparticles in Ge-espun is highlighted in Table 1. The EDX pattern of elements detected in nanofibers is available as Supplementary Data in Appendix A.

Table 1. Quantitative EDX analysis of the presence of nanometals in the Ge-espun nanofibrous matrix

Metal nanoparticles / Ge-espun	Elements (w/w)%				
	Ag	**Cu**	**Fe**	**Ni**	**Zn**
Neat-espun	-	-	-	-	-
Ag/Ge-espun	0.41	-	-	-	-
Cu/Ge-espun	-	0.44	-	-	-
Fe/Ge-espun	-	-	0.42	-	-
Ni/Ge-espun	-	-	-	0.45	-
Zn/Ge-espun	-	-	-	-	0.41

3.3. Antimicrobial activity

The antibacterial activity is a demonstration of release of metal ions from nanofiber matrix. Metal nanoparticles exhibit relatively large surface area, thus increasing their contact with bacteria. Fig. 4 shows the results of antibacterial efficacy of Ge-espun loaded with metal nanoparticles against some common bacteria found on wounds. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25023. The antibacterial activity is based on the disc diffusion method and expressed as a ratio of clear inhibition of microorganisms in contact with all metal nanoparticles loaded on the nanofibers and the neat Ge-espun. It should be noted that the initial diameter of all the specimens was fixed at 0.7cm. The relative zone of inhibition was calculated from Eq. (4).

$$\text{Relative Zone of Inhibition} = \frac{B-A}{A} \times 100 \quad (4)$$

where *A* and *B* are the diameter of the clear inhibitory zone of the neat espun and the clear inhibitory zone of espun loaded with metal nanoparticles, respectively.

According to the data obtained, no antibacterial activity was detected for the neat Ge-espun nanofibers and Ni/Ge-espun against the bacteria tested. The poor inhibition by the Ni/Ge-espun was contradicted with the study reported by Popova et al. They found that the nickel ion complexes with ligands manifested good antibacterial activity against the tested bacteria, especially towards the Gram's positive strains [25]. In the recent years, focus on the bimetallic complexes of Ni with other metal nanoparticles has gained more attention in the area of dental materials. Argueta-Figueroa reported that the Ni-Cu complexes showed broad antibacterial spectrum and potentially being employed in dentistry for formulation of bone cements, irrigating the root canal and devices coating [26].

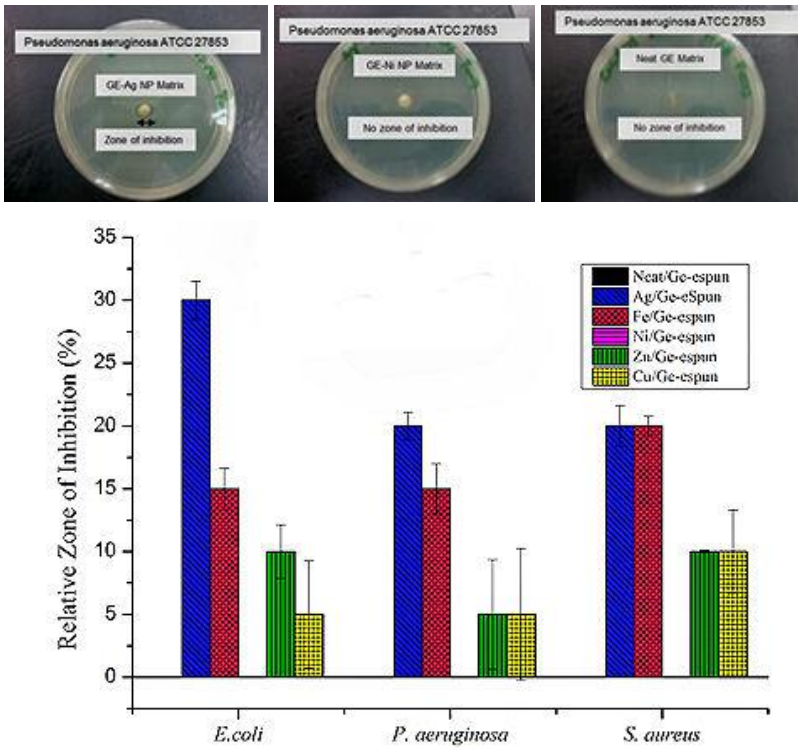

Fig. 4. The antibacterial effect of different metal loaded Ge-espun on commonly found bacteria on wounds. *Notes*: E.coli – Escherichia coli ATCC 25922; P. aeruginosa – Pseudomonas aeruginosa ATCC 27853; S. aureus – Staphylococcus aureus ATCC 25023

Evident bacteria inhibition zones were observed on the Ag/Ge-espun, Fe/Ge-espun, Zn/Ge-espun and Cu/Ge-espun. Metal nanoparticles except nickel exhibited broad spectrum antimicrobial activity. The bacterial inhibition zone on Ag/Ge-espun nanofiber was the greatest but its antibacterial efficacy reduced towards the *S. aureus* (gram positive bacteria). This can be explained by the difference in structure of the envelope of Gram-positive and Gram-negative bacteria. Gram positive bacteria have a thick peptidoglycan layer below the cell membrane that protects bacteria from external stresses and is believed to reduce the penetration of silver nanoparticle [27]. In contrast, the Fe/Ge-espun and Cu/Ge-espun displayed greater inhibition on Gram positive bacteria than Gram negative bacteria. The same pattern was also reported by Klinkajon and Supaphol [11] on the ability of the copper alginate hydrogel to inhibit Gram positive bacteria. This can be explained due to the greater abundance of amines and carboxyl groups on cell surface of Gram positive bacteria and greater affinity of copper towards these groups [28]. The copper ions are targeting the antisense that involves in gene coding and lead to DNA molecules disruption [29]. In the case of Fe/Ge-espun, the bacterial inhibition was driven by iron chelation by the chelator. Based on this concept, the higher the iron binding constant to the chelator (Ge-espun), the stronger the predicted antimicrobial activity [30]. The composition of the microorganism's cell wall and the physical nature of

the iron chelator also affect the inhibition of the bacterial growth [31]. The exact mechanism behind bactericidal effect of iron nanoparticles is not known and needs to be studied further.

4. Conclusions

In this work, copper, iron, nickel, silver and zinc were successfully impregnated into Ge-espun nanofibers using electrospinning technique from 1% (v/v) solutions of their salts precursor and 18% (w/v) of gelatin solution. The presence of the metal nanoparticles was confirmed using the surface plasmon absorption band and energy-dispersive X-ray (EDX) spectroscopy. The amount of metal nanoparticles increased with the increase of aging time. Both neat and metal nanoparticles/Ge-espun were spun into smooth nanofibers matrices. The average diameter of nanofiber tended to decrease with nanometals loaded fibres. The average diameter of metal nanoparticles ranged from 40nm to 150nm. Wide variation of nanofiber diameter was observed on the Ag/Ge-espun. Metal nanoparticles doped gelatin nanofibers manifested good antibacterial effects except Ni/Ge-espun. The nanocomposite of Ag/Ge-espun showed greater inhibition against the Gram's negative bacteria but exhibited lower efficacy on the Gram's positive bacteria. In contrast, the Cu/Ge-espun and Fe/Ge-espun displayed high efficacy on the Gram's positive bacteria. Bimetallic complexes of Ag-Cu doped electrospun gelatin could be explored in the future for potential metal-based antibiotics that could prevent the emergence of antibiotic-resistant bacterial strains.

Acknowledgement

The authors would like to thank the researcher in the Faculty of Engineering and the Faculty of Science, University of Nottingham Malaysia Campus for the facilities and the helpful discussion. This study was supported by a research grant (NER40002) from the Ministry of Higher Education (MOHE) and partially supported by the Faculty of Engineering, University of Nottingham Malaysia Campus.

References

1. Ziabari, M.; Mottaghitalab, V.; Haghi, A.K.; McGovern, S.T. (2009). Control of electospun nanofiber diameter using distance transform method: A novel approach. *Electrospun nanofibers and nanotubes research advances*, A. Haghi, ed., Nova Science Publishers, Inc, New York, 69-94.
2. Teo, W.E.; Ramakrishna, S. (2006). A review on electrospinning design and nanofibre assemblies. *Nanotechnology*, 17(14), R89-R106.
3. Fong, H., Chun, I.; Reneker, D. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, 40(16), 4585-4592.
4. Schrieber, R.; Gareis, H. (2007). *Gelatine handbook: theory and industrial practice*. Wiley-VCH Verlag GmbH & Co. KGaA, Germany.
5. Schrieber, R. (2007). From collagen to gelatin. *Gelatin handbook*, Wiley-VCH Verlag GmbH & Co. KGaA, Germany, 113-114.

6. Hoque, M.; Nuge, T.; Tshai, K.Y.; Nordin, N. (2013). Electrospinning of gelatin nanofibre: current trends in tissue engineering applications. *Journal of Applied Mechanical Engineering*, 2(4), 1000e122.
7. Nuge, T.; Hoque, M.; Tshai, K.Y.; Nordin, N.; Chowdhury, M. (2013). Electrospun gelatin composite nanofibres: A review on structural and mechanical characterizations. *Regenerative Research*, 2(2), 39-42.
8. Gao, Y.; Bach Truong, Y.; Zhu, Y.; Louis Kyratzis, I. (2014). Electrospun antibacterial nanofibers: Production, activity, and in vivo applications. *Journal of Applied Polymer Science*, 131(18), 40797.
9. Yang, H.; Gao, P.F.; Wu, W.B.; Yang, X.X.; Zeng, Q.L.; Li, C.; Huang, C.Z. (2014). Antibacterials loaded electrospun composite nanofibers: release profile and sustained antibacterial efficacy. *Polymer Chemistry*, 5(6), 1965-1975.
10. Mohiti-Asli, M.; Pourdeyhimi, B.; Loboa, E.G. (2014). Skin tissue engineering for the infected wound site: biodegradable PLA nanofibers and a novel approach for silver ion release evaluated in a 3D coculture system of keratinocytes and staphylococcus aureus. *Tissue Engineering Part C Methods*, 20(10), 790-797.
11. Klinkajon, W.; Supaphol, P. (2014). Novel copper (II) alginate hydrogels and their potential for use as anti-bacterial wound dressings. *Biomedical Materials*, 9(4), 45008.
12. Chaturvedi, A.; Bajpai, A.K.; Bajpai, J.; Sharma, A. (2015). Antimicrobial poly(vinyl alcohol) cryogel–copper nanocomposites for possible applications in biomedical fields. *Designed Monomers and Polymers*, 18(4), 385-400.
13. Lin, Y.H.; Hsu, W.S.; Chung, W.Y.; Ko, T.H.; Lin, J.H. (2014). Evaluation of various silver-containing dressing on infected excision wound healing study. *Journal of Materials Science: Materials in Medicine*, 25, 1375-1386.
14. Prabhu, S.; Poulose, E.K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters*, 2(32), 1-10.
15. Tian, J.; Wong, K.K.Y.; Ho, C.-M.; Lok, C.N.; Yu, W.Y.; Che, C.M.; Chiu, J.F.; Tam, P.K.H. (2007). Topical delivery of silver nanoparticles promotes wound healing. *ChemMedChem*, 2(1), 129-136.
16. Shin, S.H.; Ye, M.K.; Kim, H.S.; Kang, H.S. (2007). The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells. *International Immunopharmacology*, 7(13), 1813-1818.
17. Bonnemann, H.; Richards, R.M. (2001). Nanoscopic metal particles - synthetic methods and potential applications. *European Journal of Inorganic Chemistry*, (10), 2455-2480.
18. Upadhyaya, M.; Ahmed, N.; Deka, R.; Kakati, D.K. (2012). Studies on Cu^{2+} ion doped polyaniline. *Iranian Polymer Journal*, 21(9), 601-607.
19. Sharma, V.K.; Yngard, R.A.; Lin, Y. (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science*, 145(1-2), 83-96.
20. Jeong, L.; Park, W.H. (2014). Preparation and characterization of gelatin nanofibers containing silver nanoparticles. *International Journal of Molecular Sciences*, 15(4), 6857-79.

21. Haghi, A.; Zaikov, G. (2011). Polymeric nanofibre fabrication via electrospinning process. *Advances in Nanofibre Research*, Shropshire: Smithers Rapra, 11-39.
22. Hoque, M.E.; Nuge, T.; Tshai, K.Y.; Nordin, N.; Prasad, R.G.S.V. (2014). Gelatin electrospun nanofibre scaffold for potential wound healing application. *9th International Materials Technology Conference and Exhibition*.
23. Madhumathi, K.; Sudheesh Kumar, P.T.; Abhilash, S.; Sreeja, V.; Tamura, H.; Manzoor, K.; Nair, S.V.; Jayakumar, R. (2010). Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. *Journal of Materials Science: Materials in Medicine*, 21(2), 807-813.
24. Rujitanaroj, P.O.; Pimpha, N.; Supaphol, P. (2008). Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. *Polymer*, 49(21), 4723-4732.
25. Popova, T.P.; Alexandrova, R.I.; Tudose, R.; Mosoarca, E.M.; Costisor, O. (2012). Antimicrobial activity in vitro of four nickel complexes. *Bulgarian Journal of Agricultural Science*, 18(3), 446-450.
26. Argueta-Figueroa, L.; Morales-Luckie, R.A.; Scougall-Vilchis, R.J.; Olea-Mejía, O.F. (2014). Synthesis, characterization and antibacterial activity of copper, nickel and bimetallic Cu–Ni nanoparticles for potential use in dental materials. *Progress in Natural Science: Materials International*, 24(4), 321-328.
27. Quirós, J.; Borges, J.P.; Boltes, K.; Rodea-Palomares I.; Rosal, R. (2015). Antimicrobial electrospun silver-, copper- and zinc-doped polyvinylpyrrolidone nanofibers. *Journal of Hazardous Materials*, 299, 298-305.
28. Ruparelia, J.P.; Chatterjee, A.K.; Duttagupta, S.P.; Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*, 4(3), 707-716.
29. Bai, H.; Sang, G.; You, Y.; Xue, X.; Zhou, Y.; Hou, Z.; Meng, J.; Luo, X. (2012). Targeting RNA polymerase primary σ 70 as a therapeutic strategy against methicillin-resistant Staphylococcus aureus by antisense peptide nucleic acid. *PLOS One*, 7(1), 1-10.
30. Qiu, D.-H.; Huang, Z.-L.; Zhou, T.; Shen, C.; Hider, R.C. (2011). In vitro inhibition of bacterial growth by iron chelators. *FEMS Microbiology Letters*, 314(2), 107-111.
31. Chew, B.P.; Tjoelker, L.W.; Tanaka, T.S. (1985). In vitro growth inhibition of mastitis causing bacteria by phenolics and metal chelators. *Journal of Dairy Science*, 68(11), 3037-3046.

Appendix A

Supplementary Data: EDX analysis of (A) Ag/Ge-espun; (B) Cu/Ge-espun; (C) Zn/Ge-espun; (D) Fe/Ge-espun and (E) Ni/Ge-espun