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Nanofiber Scaffold Coated with Ag and ZnO Nanoparticles for Treatment of Methicillin Resistant *Staphylococcus aureus*

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Abstract Silver (Ag) nanoparticles are well established for its antibacterial activity. In this study, we demonstrate the antibacterial activity of the electrospun nanofiber mats coated with various ratios of Ag and ZnO nanoparticles and relate it with the hydrophilicity of the membrane imparted due to Ag nanoparticles. Electrospun nanofibers were prepared from a 1:1 blend of two polymers: PCL and PMMA that was sputter coated with inorganic nanoparticles (Ag and ZnO) at three ratios thus adding another layer of nanocomposition to the resulting polymer nanocompoite nanofiber scaffold. The antibacterial activity of scaffolds coated with different ratios of Ag and ZnO was tested against MRSA ATCC®. The viable bacteria were monitored by counting the number of colony forming units (CFUs/ml). The PF-QNM characterization results showed different shapes, sizes and DMT modulus of the inorganic nanoparticles (Ag and ZnO), appearing at the surface of the nanofibers. Ag and ZnO nanoparticles were observed heterogeneously distributed on the nanofiber mesh and varied at different locations along the nanofibers lengths based on their ratios used in sputtering. Increasing ZnO content increased both the hardness and water contact angle (almost double as compared to Ag for the same increase in content) of the nanofiber mesh. The results revealed a significant reduction ($p < 0.05$) in the number of CFUs/ml after only 15 min of exposure to the scaffolds coated with Ag:ZnO (1:1) and Ag:ZnO (3:1) respectively. Nevertheless, scaffold coated with Ag:ZnO (1:3) required longer time (30 min) to show reduction in the number of CFUs/ml. There was a significant difference between the number of CFUs/ml after 0 min exposure to scaffolds coated with different ratios of Ag and ZnO and the number of CFUs/ml after 30 min exposure. Taken together these results, superior antibacterial activity for scaffolds coated with different ratios of Ag and ZnO against pathogenic bacteria MRSA was reported, which demonstrates potential applications of these scaffolds in medical and biomedical fields.

Keywords: *nanofibers, nanomechanical properties, antibacterial properties, Electrospinning, MRSA*

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1. Introduction

The last decade has seen an increasing trend in designing scaffolds for tissue engineering from biodegradable polymeric fibers with nano and submicron diameters specifically produced by electrospinning method. Partly successes in using such scaffolds are attributable to structural similarity to the extracellular matrix (ECM), convenient porosity for cellular proliferation, high surface area to volume ratio, reduced immunogenicity and biodegradability among others. [1] After implantation of such scaffolds, in biomechanical environment of the body, however the fibers in the scaffolds are subjected to stresses and strains in physiological conditions, thereby standing a chance to permanent deformation or even failure to scaffold structure. Therefore, there is a pressing

need to characterize their mechanical properties, especially at the nanoscale as well as to assess resulting surface properties such as wettability.

Nano-mechanical properties of the deigned scaffold has been characterized in this study using Atomic force microscopy (AFM). AFM in the family of scanning probe microscopy has emerged as a promising tool with a host of powerful techniques that have been employed in imaging of the components of biomaterials scaffolds and probing selected mechanical properties under physiological conditions. [2,3,4] The PeakForce Quantitative Nano Mechanics (PF-QNM) is relatively a new addition to the host of techniques allowing to measure nano-mechanical properties of the materials including reduced Young`s modulus, adhesion, dissipation, and deformation concurrently with topographical height imaging. [5]

Wettability pertains to the interaction between solid and fluid phases at the interface. Strictly the contact angle of the fluid with the solid phase at which the liquid–vapor interface meets the solid–liquid interface, explains the wettability. This in turn is determined by a force balance between adhesive and cohesive forces. An increasing tendency of a drop to spread out over a flat, decreases the contact angle, thereby providing an inverse measure of wettability. A contact angle less than 90° (low contact angle) usually indicates that wetting of the surface is very favorable, and that the surface is hydrophilic while a contact angles greater than 90° (high contact angle) commonly means that wetting of the surface is unfavorable and that the surface is hydrophobic.

Infections of the wound are considered as common life-threatening worldwide problem resulting in 300,000 death every year. [7] A wound is a break in the skin, leading to exposure of subcutaneous tissue due loss of skin integrity. [8] It varies among patients from acute, chronic, burn and traumatic wounds. [9] The infection causes delayed healing, longer stay in the hospital and increased the economic pressure. [8] Wounds are vulnerable to be contaminated with different microbes, which capable of causing infection. [10] Many factors enhance the bacterial colonization of wounds such as origin, body location, size and duration of wounds. [9] Most common species colonize wounds are Methicillin-resistant *Staphylococcus aureus* (MRSA) followed by *E. coli*, and *P. aeruginosa*. [11,12] For the past several decades, clinicians used to prescribe antibiotics such as β-lactam, vancomycin, daptomycin and rifampicin for treatment of wound infection. [13] Thus, the improper using of antibiotics leads to the emergence and dissemination of multidrug-resistant bacteria (MDR). [14] Nowadays, emerging of antibacterial resistance pathogenic strains become a global health challenge. [9] In this era, researchers start to look for alternative treatments and replaced the conventional antibiotics for the treatment of MDR. [15] Metallic nanoparticles (NPs) such as silver (Ag), gold (Au), titanium (Ti), zinc (Zn), Magnesium (Mg), Aluminum (Al) and copper (Cu) have demonstrated antibacterial activity against several pathogenic microorganisms. [15,16,17] Recently, nanobiotechnology has offered great possibilities of these nanomaterials to be employed as alternatives to antibiotics for the treatment of MDR. [18]

In this study, we demonstrated the antibacterial activity of the electrospun nanofiber mats coated with various ratios of Ag and ZnO nanoparticles. The nano-mechanical properties and the wettability of the nanofiber have been accomplished and demonstrated. Superior antibacterial activity of the nanofibers coated with different ratios of Ag and ZnO against pathogenic bacteria MRSA was reported, which demonstrates potential applications of these scaffolds in medical and biomedical fields especially in wound dressing.

2. Materials and Methods

2.1. Fabrication of the Nanofibers

The nanofiber was fabricated using two polymers namely Polycaprolactone (PCL) and poly methyl methacrylate (PMMA). The two polymers were blended in 1:1 ratio to make base nano-composite fibers, produced by electrospinning from solution at applied voltage of 12 kV. Thereafter, this was transformed into three different kinds of nanofiber meshes through sputtering of Ag and ZnO in three different ratios: a) 30:30, b) 30:100 and c) 100:30 of Ag:ZnO. The sputtering was performed using RF Sputtering System following the optimized protocol at the nanotechnology central facility of King Abdulaziz University.

2.2. Nano-mechanical Characterization of the Nanofibers

Bruker Dimension Icon AFM (Bruker Corporation, CA, USA) with ScanAsyst™ was used to determine modulus of the fibers in the scaffold as well to image simultaneously the surface topographical information as height sensor mages. The recording of all images was performed in the PeakForce QNM (Quantitative NanoMechanics) imaging mode using typical silicon tips (TESPA, spring constant 20-80 N/m, Veeco, Santa Barbara, CA, USA). Imaging was conducted in air under ambient conditions. The DMT modulus were determined as per the method reported previously. [6] A typical output of the DMT modulus analysis is presented in Figure 1. DMT modulus Rms values were calculated across each line cross section of the DMT modulus map as shown in Figure 1. At least three such cross-sectional values of DMT modulus were averaged across each mapping done on the fibers. Scanning Electron Microscope (SEM) was used to monitor the coating and the composition of Ag and ZnO nanoparticles on the nanofibers. Furthermore, Energy Dispersive X-ray Spectroscopy (EDS) was used to provide a rapid quantitative analysis of Ag and ZnO composition.

2.3. Water Contact Measurements of the Nanofibers

The water contact angle was measured following a standardized protocol in the biomaterials and tissue engineering laboratory, ECE department at King Abdulaziz University. Briefly, drops of double-distilled water were measured at room temperature (25°C) using Attension Optical Tensiometer Theta 200 (Biolin Scientific, Stockholm, Sweden) equipped with a CCD

camera, frame grabber, and image analysis software. θ values were determined on the coated nanofiber mat by mounting it on a microscope slide with double-sided adhesive tape. Two-microliter drops of water was deposited onto the fiber mat surfaces with a computer software controlled dosing system holding a 1mL syringe with a 0.5-mm-diameter needle as shown in the Figure 3 in the result section. Side-view images of the drops were captured at a rate of 6 frames s^{-1}. θ values were automatically calculated by fitting the captured drop shape to the one calculated from the Young-Laplace equation using the software integrated the equipment.

2.4. Antibacterial Actvity Studies

Staphylococcus aureus ATCC® 43300MINIPACK™ (MRSA) was grown overnight in 250 ml Erlenmeyer flask containing 100 ml Luria Bertani (LB) broth, and incubated in 150 rpm orbital shaker incubator at 37°C. The overnight culture was harvested by centrifugation at 6000 rpm for 5 minutes, and washed twice with normal saline (0.85% NaCl solution at pH 6.5) to remove any bound organic and inorganic components. The washed cells were re-suspended in 4 ml normal saline. Scaffolds coated with different ratios of Ag and ZnO were washed with 70% ethanol and left to dry for 5 min. The bacterial suspension was exposed to scaffolds coated with different ratios of Ag and ZnO and incubated at 37°C in 150 rpm orbital shaker incubator. An aliquot (100 µl) was taken at different time intervals (0, 30, 60 min for MRSA), diluted and plated on LB agar plates to determine the number of colony forming units (CFUs/ml). Counts of CFUs were performed by applying the drop-plate method. Serial dilutions (1:10) were achieved by diluting the bacterial cells (100 µl) in microcentrifuge tubes containing 25% normal saline (900 µl). Three 10 µl aliquots of the appropriate dilution were spotted onto LB agar plate and incubated overnight at 37°C. The experiment was performed in triplicate, and negative control (no scaffolds incubated with MRSA) was used.

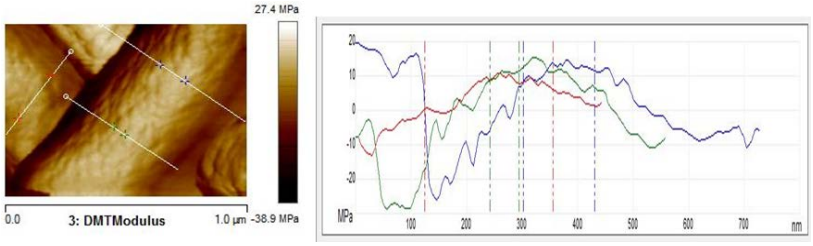


Figure 1. A typical DMT modulus calculation output of the NanoScope Analysis software. Each DMT modulus map was analyzed at least using three cross sections across the modulus map

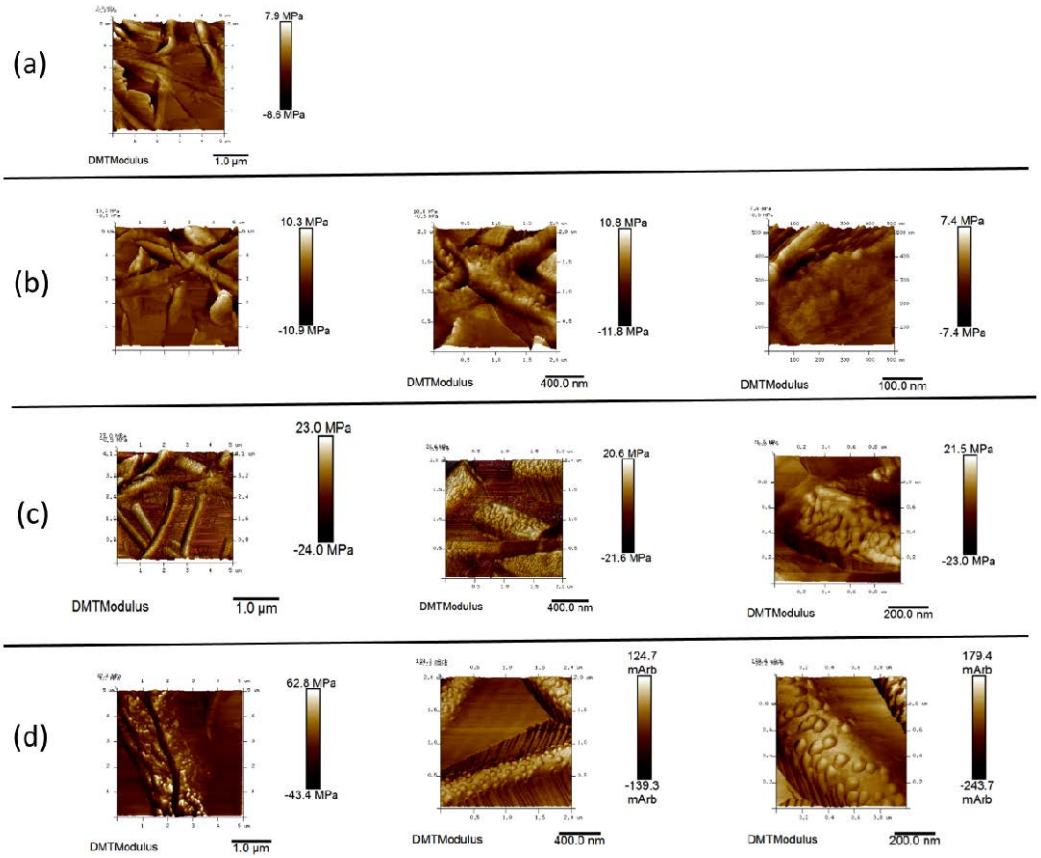


Figure 2. AFM DMT modulus images. High magnification DMT modulus images of the fiber surface clearly shows morphological appearance of the two, Ag and ZnO nanoparticles in micrograph. (a) Non-coated background fiber mat; (b) 30:30 (Ag:ZnO) sputtered fiber mat; (c) 100:30 (Ag:ZnO) sputtered fiber mat; (d) 30:100 (Ag:ZnO) sputtered fiber mat

3. Results and Discussion

3.1. Nano-mechanical Characterization of the Nanofibers

Figure 2 shows the micrographs of PCL:PMMA electrospun nanofiber meshes sputtered with three combinations of Ag:ZnO obtained by AFM operating in PF-QNM mode. Our repeated experiments produced reproducible results as reported earlier elsewhere. [6] The high magnification DMT Modulus images in (c) of Ag:ZnO combination of 100:30 sputtering show predominantly Ag nanoparticles (relatively smaller sizes) while mages in (d) of Ag:ZnO combination of 30:100 sputtering show predominantly ZnO nanoparticles (relatively larger sizes). The later combination shown in (d) of Figure 2 corresponds to higher values of Young modulus [6] as compared to 100:30 combination of Ag:ZnO as shown in (c) of Figure 2. The corresponding height sensor images (larger size) as reported earlier do not have distinct information regarding Ag and ZnO nanoparticle anchored on the fiber surfaces as a result of the sputtering method. [6]

3.2. Water Contact Angel of the Nanofibers

The water contact angle values doubled (16%) for Ag:ZnO combination of 30:100 as compared to 100:30 combination of Ag:ZnO which increased by 8% from the value exhibited by 30:30 combination of Ag:ZnO. The average values of water contact angles of three different combinations of Ag:ZnO sputtering were very near to the values reported earlier.[6] Coating of Ag nanoparticles imparted hydrophilicity to the nanofibers mats used in this study while increasing the ratio of ZnO added to the enhanced hydrophobicity. Hydrophilic surfaces are more prone to adhesions of materials and molecules thereby bacteria can easily adhere to such surfaces. Increased adhesion of bacteria to the graded hydrophilic Ag coated electrospun fiber mats based on various ratios used should inflict lethality to the adherent bacteria accordingly. With these in mind, we used the prepared membrane for antibacterial activity with *Staphylococcus aureus* ATCC® 43300MINIPACK™.

Figure 3. Water contact angle measurements for the nanofibers

3.3. SEM and EDS Results

Scanning Electron Microscope (SEM) images for the nanofibers scaffold are illustrated in Figure 4. The results clearly indicated that the surface of the non-coated nanofibers was clear from any Ag and ZnO nanoparticles (Figure 4 a). On the other hand, SEM images illustrated that the nanofibers scaffold was successfully and effectively coated with different ratios of Ag and ZnO nanoparticles as presented in Figure 4 (b), (c), and (d) respectively. To provide a rapid quantitative analysis of Ag and ZnO composition, Energy Dispersive X-ray Spectroscopy (EDS) was used and the results are presented in Figure 5 and Figure 6. There was no peaks for Ag and ZnO in the non-coated nanofibers scaffold as illustrated in Figure 5. Nevertheless, Figure 6 showed two peaks of ZnO and Ag for scaffold coated with 30:100 Ag:ZnO nanoparticles, which confirms that the nanofibers scaffold surface comprise Ag and ZnO nanoparticles.

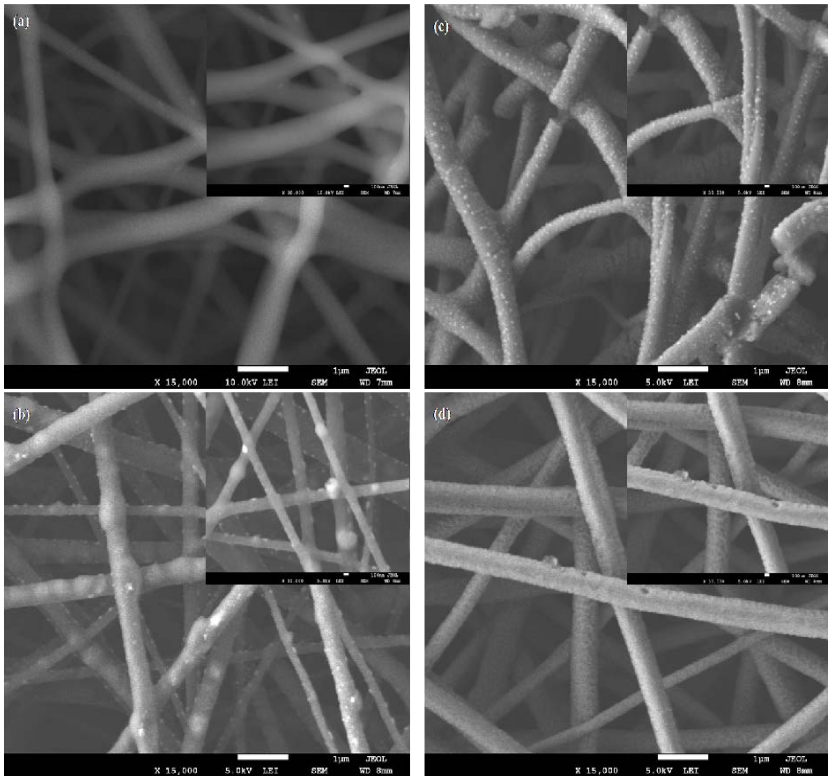


Figure 4. Scanning Electron Microscope (SEM) images for the nanofibers scaffold, (a) is the non-coated scaffold (negative control), (b) scaffold coated with 30:30 Ag:ZnO, (c) scaffold coated with 100:30 Ag:ZnO and (d) scaffold coated with 30:100 Ag:ZnO

Spectrum processing :

Peak possibly omitted : 1.750 keV

Processing option : All elements analyzed (Normalised)

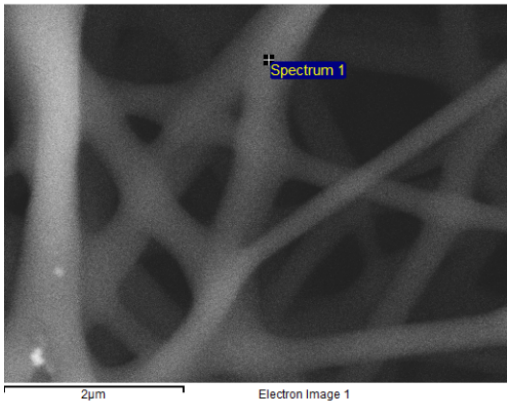
Number of iterations = 2

Standard :

C CaCO3 1-Jun-1999 12:00 AM

O SiO2 1-Jun-1999 12:00 AM

Element	Weight%	Atomic%
C K	88.97	91.49
O K	11.03	8.51
Totals	100.00	



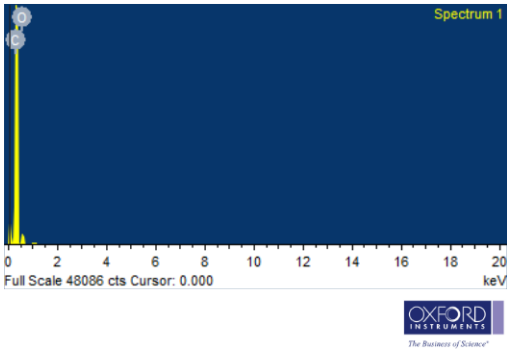


Figure 5. Energy Dispersive X-ray Spectroscopy (EDS) results for the non-coated nanofibers scaffold (negative control)

Spectrum processing :

No peaks omitted

Processing option : All elements analyzed (Normalised)

Number of iterations = 2

Standard :

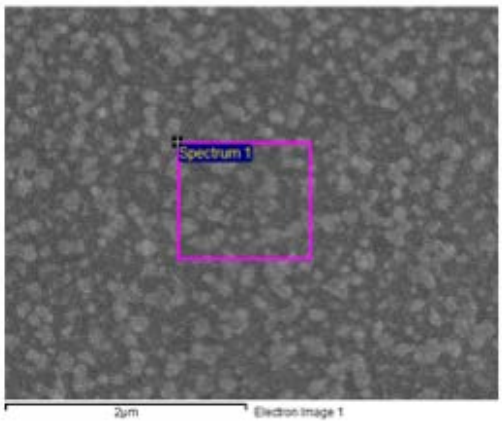
C CaCO3 1-Jun-1999 12:00 AM

O SiO2 1-Jun-1999 12:00 AM

Zn Zn 1-Jun-1999 12:00 AM

Ag Ag 1-Jun-1999 12:00 AM

Element	Weight%	Atomic%
C K	2.91	9.95
O K	18.75	48.23
Zn L	48.09	30.27
Ag L	30.26	11.54
Totals	100.00	



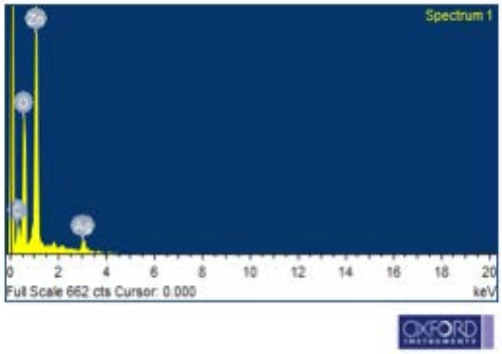


Figure 6. Energy Dispersive X-ray Spectroscopy (EDS) results for nanofibers scaffold coated with Ag and ZnO (30:100)

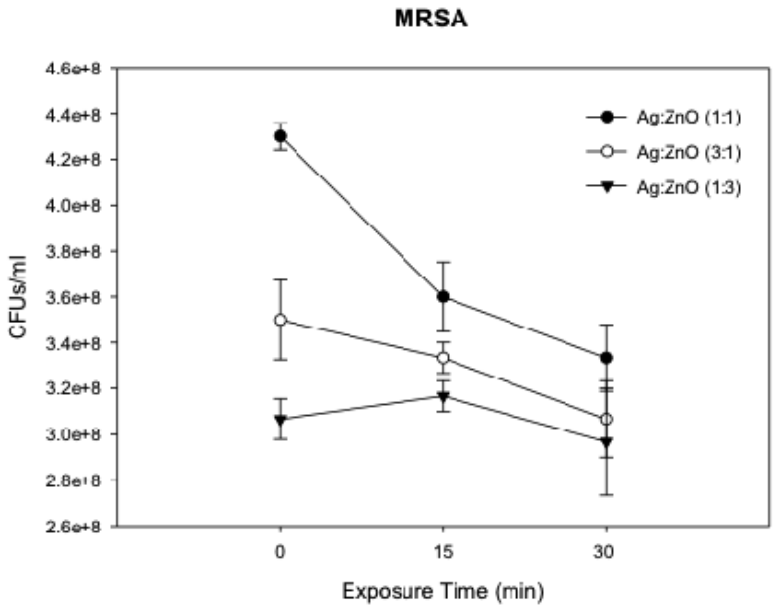


Figure 7. Antibacterial activity of scaffolds coated with different ratios of Ag and ZnO tested against *Staphylococcus aureus* ATCC® 43300MINIPACK™ at different time intervals

3.4. Results of Antibacterial Activity

The antibacterial activity of scaffolds coated with different ratios of Ag and ZnO was tested against *Staphylococcus aureus* ATCC® 43300MINIPACK™ and presented in Fig 7. The viable bacteria were monitored by counting the number of colony forming units (CFUs/ml). The presented results are an average of triplicate measurements. The results revealed a significant reduction ($p < 0.05$) in the number of CFUs/ml after only 15 min of exposure to the scaffolds coated with Ag:ZnO (1:1) and Ag:ZnO (3:1) respectively (Fig 7). Nevertheless, scaffold coated with Ag:ZnO (1:3) required longer time (30 min) to show reduction in the number of CFUs/ml (Figure 7). There was a significant difference between the number of CFUs/ml after 0 min exposure to scaffolds coated with different ratios of Ag and ZnO and the number of CFUs/ml after 30 min exposure. Taken together these results show superior antibacterial activity for scaffolds coated with different ratios of Ag and ZnO against pathogenic bacteria MRSA, which demonstrates potential applications of these scaffolds in medical and biomedical fields.

4. Conclusion

We successfully fabricated a scaffold coated with different ratios of Ag and ZnO, and use it against MRSA. A polymer blend of PCL/PMMA was used to fabricate the scaffold. PMMA was selected because it is biocompatible with human tissue, it has been extensively used in several biotechnology and biomedical research to create microfluidic lab-on-a-chip devices and it has several applications in cosmetic surgery. PCL was used to increase the elasticity of PMMA, to make a compatible environment between PMMA and water which makes the majority of human body, it is biodegradable in physiological conditions (such as human body) and it has been approved by FDA in specific applications used in human body (i.e. drug delivery device). We have obtained the nanomechanical mapping on a polymer blend of PCL/PMMA sputtered with inorganic nanoparticles. The results show superior antibacterial activity for scaffolds coated with different ratios of Ag and ZnO against pathogenic bacteria MRSA. This demonstrates potential applications of these scaffolds in medical and biomedical fields. The introduced scaffold might be an ideal biomaterial for wound dressing applications.

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