Comparative activity of silver based antimicrobial composites for urinary catheters

Authors: Nikhil Thokala[1, 5], Dr Carmel Kealey[2, 4], Dr James Kennedy[3], Dr Damien B. Brady[2, 4], Dr Joseph Farrell[1, 5]. Dept. of Mechanical and Polymer Engineering[1], Dept. of Life & Physical Science[2], Bioscience Research Institute[4], Materials Research Institute[5], Athlone Institute of Technology, Athlone and Kastus[3],Grangegorman, Ireland.

Corresponding author Dr Damien B Brady

Contact Details

Email dbrady@ait.ie

Tel 00353906468080

Abstract

Keywords: Elemental silver; Ionic silver; Glass carrier; Polymer/antimicrobial composites; Antimicrobial efficacy; Silver ion release.

Biomedical polymers are an integral component in a wide range of medical device designs due to their range of desirable properties. However, extensive use of polymer materials in medical devices have also been associated with an increasing incidence of patient infections. Efforts to address this issue have included the incorporation of antimicrobial additives for developing novel antimicrobial polymeric materials. Silver with its high toxicity towards bacteria, oligodynamic effect and good thermal stability has been employed as an additive for polymeric medical devices. In the present study, commercially available elemental (Biogate) and ionic (Ultrafresh 16) silver additives were incorporated into a Polyamide 11 (PA 11) matrix using a compression press. These polymer composites were evaluated for their antimicrobial and ion release properties. Elemental silver composites were determined to retain their antimicrobial properties for extended periods and actively release silver ions for 84 days; whereas the ionic silver composites lost their ion release activity and therefore antibacterial activity after 56 days. Bacterial log reduction units of 3.87 for ionic silver and 2.41 for elemental silver was identified within 24 hr, when tested in accordance with ISO 22196 test standard; indicating that ionic silver is more efficient for short-term applications compared to elemental silver.

1. Introduction

Antimicrobial activity of metallic silver is well established. The use of silver compounds in many different forms including sutures, solutions and colloids to treat a range of infectious diseases was commonplace until the mid-1930s. With the advent of penicillin and other antibiotics, there was a rapid decline in the use of silver and related products for clinical and disinfectant purposes. However, there has been a renewed interest in silver as an antimicrobial agent due to the emergence of the antibiotic resistant strains of bacteria including methicillin-resistant *staphylococcus aureus* (MRSA) and vancomycin-resistant *staphylococcus aureus* (VRSA) and their associated devastating nosocomial infections [1]. Public expectation for higher standards in infection prevention and control, has led to a demand for materials capable of killing pathogens on common touch surfaces and on medical device surfaces in hospitals and long-term care facilities. The most significant hospital-acquired infections, based on frequency and potential severity, are those related to the use of devices such as intravascular [2] and urinary catheters [3, 4]. While other compounds with antimicrobial properties are either too volatile or do not withstand thermal processing; noble earth metals like silver and copper, with their excellent thermal and chemical stability, toxic properties towards bacteria at low concentrations have inevitably led to their use as antimicrobial agents in the design of polymeric medical devices [5].

Elemental silver is relatively inert towards bacteria, ionisation of the elemental silver in the presence of oxygen and moisture results in the release of silver ions, which interacts with the bacterial cell wall surface components to exert their toxicity [6]. In contrast, ionic silver additives comprise a host structure capable of housing silver cations that are released through interaction with moisture [7]. These systems derive their activity on the ability to supply, under the right circumstances, a critical concentration of silver cations necessary for an antimicrobial effect. Silver, in both elemental and ionic form is widely available as an antimicrobial additive. Figure 1 presents commercially available forms of silver antimicrobial additives, including the additives selected for this study.

Silver ions are effective against nearly all pathogens of concern in healthcare environments, including *Staphylococcus aureus*, *Escherichia coli* and multi drug resistant bacterial strains [8]. Silver ions act by strongly binding to critical biological molecules like

proteins, DNA, RNA and disrupting their functions [9]. The mechanisms by which silver particles exert antimicrobial activity begin with the release of silver ions. Binding of silver ions to cell membranes and intracellular absorption is an important first step. Silver ions bind strongly to electron receptors, notably disulphide, amino, carbonyl and phosphate residues on membranes leading to intracellular absorption by phagocytosis [10-14].

Different methods for the incorporation of silver additive include *in situ* polymerization, direction deposition onto polymer surface and incorporation of the antimicrobial additive into the bulk polymer [15]. To ensure predictability and control of silver ion release, direct incorporation of the antimicrobial into the bulk polymer during the medical device manufacturing stage is likely to be, long term, a far more effective approach. This more direct approach relies on the delivery of minute quantities of ionic metal to the bacterial cell membrane.

The primary aim of this study was to critically examine elemental and ionic silver additives and understand their behaviour under specific test conditions for 80 days, a much extended period of time than previously conducted. Here we evaluate ionic and elemental silver additives for their ion release and antimicrobial properties. A medical grade Polyamide 11 (PA 11) acted as the host matrix. PA 11 is polar, aliphatic, crystalline homopolymer highly suited for medical applications. An active silver concentration of 1600 ppm was selected based on our previous studies with PA 11/copper composites [16]. 1600 ppm PA 11/silver composites were prepared by compression moulding and examined to develop novel antimicrobial materials for medical device applications such as urinary catheters, which slowly release ionic silver and mitigate bacterial colonization of polymer surfaces. There is an urgent need for antimicrobial catheters that are suitable for long-term use [17, 18]. Antimicrobial catheters that remain infection-free for up to three months could dramatically improve the quality of life of individuals trying to manage intractable urinary problems such as chronic urinary retention.

2. Materials and Powdered Masterbatch Preparation

Silver antimicrobial additives namely Biogate Hymedic 4000 (Biogate, Germany), Ultrafresh 16 (Thomson Reuters, Canada) and Biomaster GC 100 (Addmaster, UK) were selected for this study. The physical properties of these additives, as specified in the

technical datasheets as presented in Table 1. Polyamide 11 (Rilsan BMNO) a medical grade semi-crystalline polymer with a melt index value of 11.0 g/10 min was sourced form Arkema, France. Powdered Polyamide 11 was blended with these additives in a household blender at room temperature to make up a 50% w/w powder masterbatches. These uniformly blended powdered masterbatches were further compression moulded providing final active ingredient concentrations of 1600 ppm.

2.1 Elemental analysis of antimicrobial additives by Energy/Wave Dispersive X - ray spectrum (EDS) coupled with scanning electron microscope (SEM)

Elemental analysis and weight (% w/w) composition of the antimicrobial additives were determined using a Tescan Mira XMU variable pressure scanning electron microscope (SEM) in high vacuum mode coupled with Oxford EDS/WDX (energy/wave dispersive X-ray). The specimen setup was scanned between 10-20 kV at different ranges of magnification. Additional sample treatment such as surface etching or coating with a conductive layer (Gold, ~15nm thick) was applied before surface scanning to provide a path for the incident electrons to flow to ground.

2.2 Processing of Antimicrobial Formulations

2.2.1 Compression Moulding

PA 11/Ag test composites of dimensions 35 mm × 35 mm and thickness ca. 0.4 - 0.5 mm, suitable for antimicrobial efficacy and ion release studies were prepared using a Servitec Polystat 200 T compression press at 200 ^{o}C. A force of 2 kN was applied for 9 minutes and a final pressure of 38 bar was then applied for one minute.

2.3 Antimicrobial studies

Escherichia coli strain ATCC 8739, recommended for ISO 22196 test method, was obtained from MicroBioLogics Inc, USA. The test organism, stored at -80 ^{o}C on porous beads (Pro-lab diagnostics), was grown overnight in Mueller Hinton and Nutrient broth at 37 ^{o}C for determining minimum inhibitory concentration of the additives and surface antimicrobial efficacy of PA 11/silver composites respectively. The resulting bacterial suspensions were centrifuged at 10000 rpm for 10 min and the pellet resuspended in

sterile phosphate buffered saline (PBS) to give bacterial populations of 1×10^6 CFU/mL for subsequent studies.

2.3.1 Minimum Inhibitory Concentration (MIC)

The MIC for selected additives were evaluated against *E. coli* ATCC 8739 using the broth dilution method in accordance with Clinical Laboratory Standards Institute (CLSI). 100 µL of the test bacterial population was aseptically transferred into the wells of the microtiter plate and supplied with additive suspensions ranging from 0 µg/mL to 100 µg/mL. The microtitre plate was then incubated for 16-18 hr at 37 ^{o}C at a speed of 125 rpm. 100 µL of the resulting mixtures were then inoculated on to the MH agar plates and incubated for 24 hr at 37 ^{o}C. The additive concentrations showing complete reduction in bacterial colonies was recorded as the MIC as presented in Table 2.

2.3.2 Disk Diffusion assay

Susceptibility of the test organism, *E. coli* ATCC 8739 to the antimicrobial additive suspensions were determined in accordance with the Kirby Bauer method using a disk diffusion assay. Sterile disks were soaked with antimicrobial suspensions, concentrations ranging 1, 10, 100 and 500 µg/ml; these disks were then dried at 60 ^{o}C for 1 hr and aseptically transferred to the Mueller Hinton agar plates inoculated with test bacterial population. These plates were then incubated for 24 hr at 37 ^{o}C and for zones of inhibition determined. Solvent used for suspending the additives, 0.1 N HNO_3 and 30 µg/ml chloramphenicol impregnated antibiotic disks were used as negative and positive controls respectively.

2.3.3 Evaluation of antibacterial efficacy of the PA 11/Ag composites

The antimicrobial efficacy of the PA 11/Ag composites were evaluated according to the ISO 22196 standard. In brief, the square test composites (6 untreated and 3 treated with Ag additive) prepared in section 2.2.1 were placed in petri dishes and inoculated with 200 µL of the test organism. The inoculum was covered with a sterile coverslip (22 mm × 22 mm), incubated for 24 hr at 37 °C and 95% relative humidity for 3 of the 6 untreated composites and 3 composites treated with silver additive. Test composites were supplied with 10 ml of soyabean casein digest lecithin polysorbate broth (neutralising solution) and ultra-sonicated for 5 min to recover bacteria from surface of the specimens. Remaining 3

untreated composites were also processed in this manner prior to the incubation to provide comparative baseline data. Subsequently the recovered bacterial cell suspension was serially diluted in physiological saline. Petri dishes containing plate count agar were inoculated with these recovered bacterial dilutions in duplicates. These plates were incubated for 40-48 hr at 37 °C, after which colony forming units were determined.

2.4 Silver ion release kinetics and long-term antimicrobial activity

Ion release studies were carried out using Varian Atomic Absorption Spectrometer. Initially a standard curve was drawn for the 1000 mg/L Ag standard solution obtained from Sigma Aldrich, Ireland. Ion release kinetics were measured for 1600 ppm PA 11/ antimicrobial composites by immersing 1 gm of the test composite in 100 ml aqueous mixture (95 ml d. H_2O and 5 ml 0.1 N HNO_3) at 37 ^oC. The immersion liquid was recovered after 48 hr and thereafter every week, for 8 weeks and quantitatively analysed. Selected PA 11/antimicrobial composites were analysed for long term antimicrobial activity, using the recovered aqueous mixture, similar to the method used for determining the MIC.

3. Results and Discussion

3.1 Elemental analysis by EDS (Energy/Wave Dispersive X - ray spectrum)/SEM

As shown in Figure 2 elemental silver (Biogate) comprises ~95% (w/w) Ag and ~5% (w/w) carbon. Ionic silver additives, Ultrafresh 16 and Biomaster comprises carbon, oxygen, magnesium, silicon, phosphorous, silver and tungsten. As expected oxygen was found to be the major element followed by phosphorous and carbon. Small quantities of aluminium was identified only in Biomaster. The elements aluminium, magnesium and silicon in the form of their respective oxides acts as the inorganic carrier for the silver ions. Approximately 0.89% (w/w) and 1.28% (w/w) of silver was determined from the elemental analysis of Ultrafresh 16 and Biomaster respectively. Tungsten, which acts as a radio opacifying agent was also observed to be in equal amounts to that of silver in these ionic silver additives.

3.2 Determination of MIC

When tested against *E. coli* ATCC 8739 at a working bacterial population of 1×10^6 CFU/mL, it was observed that porously designed elemental silver was more effective even at lower concentrations compared to ionic silver. The porous nature and relatively larger surface area of the elemental silver examined might be responsible for its effective bacterial inhibitory activity. The MIC for elemental silver additive was determined to be 1µg/ml whereas, MIC for ionic silver additives Biomaster and Ultrafresh 16 were 10 and 20 µg/ml respectively as displayed in Table 2. As there was no real difference in MIC for Ultrafresh and Biomaster, antimicrobial efficacy and ion release studies were continued only with Ultrafresh.

3.3 Disk Diffusion

Disk diffusion studies show that *E. coli* ATCC 8739 was susceptible towards ionic antimicrobial additives. Biomaster and Ultrafresh 16 could diffuse through the agar and exhibited clear zones of inhibition. The elemental silver, Biogate did not show zones of inhibition; which may suggest an inferior antibacterial activity on agar plates, as compared to that in liquid medium, as identified from its MIC. It is likely that restricted mobility of the silver ions from the elemental silver additive through the semi-solid agar resulted in its failure to show zones of inhibition. As shown in Figure 3, a maximum of 12.5 mm and 13 mm diameter zones of inhibitions were observed at 100 µg/ml silver ion concentration for Biomaster and Ultrafresh 16 respectively, which were less compared to the 30 µg/ml chloramphenicol impregnated disks.

3.4 Antimicrobial efficacy of PA 11/silver composites

Surface antimicrobial properties for PA 11/ silver composites were determined in accordance with our previous studies on PA11/copper composites [16]. Test composites with a common active agent loading of 1600 ppm were examined for their antimicrobial efficacies, against a test population of 1×10^6 CFU/mL *E. coli* ATCC 8739 strain. Untreated PA 11 was used as control sample. Figure 4 depicts that untreated PA 11 had no antimicrobial effect, after 24 h exposure the number of viable bacteria increased from 1×10^6 CFU/mL to 2.94×10^6 CFU/mL. As per the test standard ISO 22196, to consider an antimicrobial system to be effective in eliminating the test bacteria, it must generate log reduction values in the range of ≥ 2 log units [19]. As presented in Figure 4 the log reduction values were 3.87 and 2.41 for ionic silver (Ultrafresh 16) and elemental silver

(Biogate) composites respectively; indicating the bacterial reductions in the range of 99.9% to >99.9% within 24 hr.

3.5 Ion release and long-term antimicrobial efficacy for PA 11/silver composites

The ion release rates for the antimicrobial composites were determined weekly, over an 8-week period. A stable release of silver ion was observed for composites with elemental silver as shown in Figure 5. Few ions were released initially, with a more controlled ion release pattern observed over the course of time suggesting a long-term ion release capability for elemental silver composites. After 7 days, the number of ions released from elemental silver composites could reduce almost 99% of bacterial population. Thereafter >99% reduction in bacterial population was observed for days 14, 21, 28 and 35 with 37, 42, 39 and 32 µg/l/g silver ions released into the aqueous mixture. Interestingly a burst release of silver ions was observed for ionic silver composites. 150 µg/l/g silver ions were released within 7 days and the corresponding bacterial reduction values were observed, indicating the number of ions released were directly proportional to the percentage reduction in bacterial population. However, the number of ions released from these ionic systems declined after 7 days and ceased altogether after approximately 56 days. Kumar *et al.*, also compared the ion release properties of PA6 composites for a shorter time frame with elementary silver and ionic silver in a carrier and concluded that some of the ionic silver additives showed the burst release effect initially and gradually became inefficient [20]. The polar nature of PA 11 allows diffusion of water molecules into the matrix, resulting in a burst release effect within 2 days for these systems. Although comparable bacterial log reduction values were observed for both the composite systems; elemental silver was more active against *E. coli* ATCC 8739 for extended periods. After 35 days, log reduction values of 2.4 and 1.4 were observed for elemental and ionic silver composites respectively. Furthermore, the elemental silver composites eluted biologically significant numbers of silver ions sufficient to control the bacterial populations. However this work will need to be extended to study other urinary catheter associated pathogens including *Pseudomonas. aeruginosa*, *Klebsiella pneumonia* and *Candida albicans*. In addition the *in vitro* activity of the antimicrobial composites would need to be tested in an environment simulating the urinary tract.

4. Conclusion

In this work antimicrobial susceptibility, antimicrobial efficacy and ion release kinetics of PA 11/silver composites were examined. Ionic silver additive could diffuse through the agar to show zones of inhibition, whereas the elemental silver additive failed to diffuse through agar medium. However, when incorporated into PA 11 and evaluated for polymer surface antimicrobial efficacy in accordance with ISO 22196 standard, both the composite systems were active against *E. coli* ATCC 8739. Bacterial reductions of >99% were observed for these composites within 24 hr. A controlled delivery of silver ions was observed for elemental silver composites with extended ion release profiles. The coinciding long-term antimicrobial efficacy for these composites suggest elemental silver composites ideally suits long-term catheterization. In contrast, ionic silver composites are more suitable for shorter term use to prevent microbial attachment or growth on a catheter surface with a burst release of silver ions within 48 hr.

Acknowledgements

The authors are grateful to Innovative Polymer Compounds (IPC) Ireland for providing PA 11 materials and for permission to use their moulding facilities.

References

1. Peng JJY, Botelho MG & Matinlinna JP. Silver compounds used in dentistry for caries management: a review. *Journal of Dentistry*, 2012; 40, 521-41.

2. Daneshpour N, Collighan R, Perrie Y, Lambert P, Rathbone D, Lowry D & Griffin M. Indwelling catheters and medical implants with FXIIIa inhibitors: A novel approach to the treatment of catheter and medical device-related infections., *Eur. J. Pharm. Biopharm*, 2013; 83, 106–13.

3. Cooper IR, Pollini M & Paladini F. The potential of photo-deposited silver coatings on Foley catheters to prevent urinary tract infections. *Materials Science & Engineering. C, Materials for Biological Applications*, 2016; 69, 414–20.

4. Fisher LE, Hook AL, Ashraf W, Yousef A, Barrett D, Scurr DJ & Bayston R. Biomaterial modification of urinary catheters with antimicrobials to give long-term broadspectrum antibiofilm activity. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, 2015; *202*, 57–64.

5. Palza H. Antimicrobial polymers with metal nanoparticles. *International Journal of Molecular Sciences*, 2015; *16*(1), 2099–116.

6. Damm C, Munstedt H & Rosch A. The antimicrobial efficacy of polyamide 6/silver-nano- and microcomposites. *Materials Chemistry and Physics*, 2008;108, 61-66.

7. Anupharb SS, Srikhirin T, Thaweboon B, Thaweboon S, Amornsakchai T, Dechkunakorn S & Kamaguchi A. Antimicrobial effects of silver zeolite, silver zirconium phosphate silicate and silver zirconium phosphate against oral microorganisms. *Asian Pacific Journal of Tropical Biomedicine*, 2013; *3*(1), 47–52.

8. Honda M, Kawanobe Y, Ishii K, Konishi T, Mizumoto M, Kanzawa N, Matsumoto M & Aizawa M, In vitro and in vivo antimicrobial properties of silver-containing hydroxyapatite prepared via ultrasonic spray pyrolysis route., *Materials Science & Engineering. C, Materials for Biological Applications*, 2013; 33, 5008–18.
9. Tran PA, Hocking DM & O'Connor AJ. In situ formation of antimicrobial silver nanoparticles and the impregnation of hydrophobic polycaprolactone matrix for medical device applications. *Materials Science & Engineering. C, Materials for Biological Applications,* 2015; 47, 63-69.
10. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, De Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *International Journal of Antimicrobial Agents*, 2009; *34*(2), 103–10.
11. Rai M, Yadav A & Gade A, Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 2009; 27, 76–83.
12. Lansdown AB. A Pharmacological and Toxicological Profile of Silver as an Antimicrobial Agent in Medical Devices. *Advances in Pharmacological Sciences*, 2010.
13. Morones-Ramirez JR, Winkler JA, Spina CS & Collins JJ. Silver enhances antibiotic activity against gram-negative bacteria. *Science Translational Medicine*, 2013; 5(190), 190ra81.
14. McEvoy JG & Zhan Z. Antimicrobial and photocatalytic disinfection mechanisms in silver-modified photocatalysts under dark and light conditions. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, 2014; *19*, 62–75.
15. Sedlarik V. Antimicrobial modification of polymers:chapter 7, *Intech*, 2013.
16. Thokala N, Kealey C, Kennedy J, Brady DB & Farrell J. Characterisation of polyamide 11/ copper antimicrobial composites for medical device applications. *Materials Science & Engineering. C, Materials for Biological Applications*, 2017; 78, 1179-1186.
17. Tenke P, Kovacs B, Bjerklund Johansen TE, Matsumoto T, Tambyah PA, Naber KG. European and Asian guidelines on management and prevention of catheter-associated

urinary tract infections. *International Journal of Antimicrobial Agents*, 2008; 31 (1), 68–78.

18. Zhang W, Liu A, Hu D, Xue D, Li C, Zhang K & Ma H. Indwelling versus Intermittent Urinary Catheterization following Total Joint Arthroplasty : A Systematic Review and meta analysis, *PLoS ONE,* 2015; 10(7), 1–13.

19. Altan M & Yildirim H. Mechanical and Antibacterial Properties of Injection Molded Polypropyle/TiO_2Nanocomposites : Effects of Surface Modification, *J. Mater. Sci. Technol.* 2012; *28*(8), 686–692.

20. Kumar R & Münstedt H. Polyamide/silver antimicrobials: effect of crystallinity on the silver ion release. *Polymer International*, 2005; *54*(8), 1180–1186.