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COMMUNICATION

Photochemical synthesis of biocompatible and antibacterial silver nanoparticles embedded within polyurethane polymers

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In-situ light initiated synthesis of silver nanoparticles (AgNP) was employed for AgNP incorporation within the polymeric matrices of medical grade polyurethane. The resulting materials showed improved antibacterial and antibiofilm activity against *Pseudomonas aeruginosa* with negligible toxicity for human primary skin cells and erythrocytes.

Synthetic polymers have played a pivotal role in the advancement of the biomedical sciences allowing, for example, the fabrication of coating agents, synthetic bio-replacements, and widely used catheters. However, biofilm indwelling still presents one of the main limitations for the safe and long-term use of biomedical devices including polymer based heart valves and intracorporeal catheters.[1, 2] This is further aggravated in immunosuppressed individuals, children and the elderly population[3-5] where catheter-related bloodstream infection (CR-BSI) can be life threatening. Thus, although avoiding contamination during the catheter insertion and post-care of the line are the cornerstone of CR-BSI prevention; improving the antibiofilm ability of intracorporeal catheters would also reduce the incidence and severity of CR-BSI.

Considerably efforts have been done in this field, where, for example, antibiotic-impregnated (e.g. minocycline/rifampicin), coated-catheters (e.g. chlorhexidine–silver sulfadiazine), and antibiotic lock devices have been fabricated (for a complete review see ref. [5]); they generally[6, 7] fail at providing dual protection against Gram (+) and (–) bacteria.[4] Although silver impregnation of polyurethane catheters has proven not to be efficient at reducing CR-BSI risks,[8] silver nanoparticles bear the potential to overcome this, as they display wide antibacterial spectra against both Gram (+) and (–) strains[9, 10] and as recently reported by members of our team, stable-silver nanoparticles (AgNP) exceed ionic silver as antibacterial agent with non-toxic side effects for primary cells.[11-14] Embedding of premade AgNP and/or in-situ thermal reduction of silver ions within water borne polyurethane matrices has been used.[15-19] However, a methodology able to form AgNP within a suitable polyurethane polymer, avoiding harsh experimental conditions, has not been reported. In the present communication, we report the light-mediated synthesis of AgNP embedded within polyurethane (PU) films (AgNP@PU). The resulting material retained the physical properties of the original PU catheter, was biocompatible for human skin fibroblasts, showed non-hemolytic activity and has proven to control the proliferation and extent of biofilm formation for the opportunistic *Pseudomonas aeruginosa* bacteria

The main steps involved in the photochemical preparation for the materials described here can be summarized, see supporting information for further details, as follows; (i) First, PU medical grade catheter (350 mg) was dissolved in THF (6.0 mL) and a solution of AgNP precursors (10 mL) containing the photoinitiator Irgacure-2959 (I-2959, 0.05 mmol), CF_3COOAg (0.05 mmol) and cyclohexylamine (0.5 mmol) were mixed in a Petri dish and left to dry for 24 h in the dark. The film was then introduced in a round bottom quartz flask with a septum. Vacumm-N_2 steps were made by means of a Schlenk line and irradiated with UVA for 60 seconds under the N_2 atmosphere. Then, the irradiated film was left for 24 hours under air in dark. Finally, the film was thoroughly washed with ethanol (50 mL x three times) with additional PBS washes (25 mL x twelve times). . Debris and by-products from the synthesis were successfully removed with sequential ethanol washes. Initial experiments carried out using different total concentrations of silver showed that only at >7.7 mg Ag/g PU, the materials had antimicrobial properties. In this communication, otherwise indicated, we will present and discuss the results obtained for the AgNP@PU containing 15 mg Ag/g PU. Further, we also tested materials containing 15 mg Ag/g of

PU prepared using different UVA exposure times of 60 and 40 s, which we have named AgNP@PU-1 and AgNP@PU-2, respectively.

Silver surface plasmon band (AgNP-SPB, see Fig. 1) was clearly observed 24 h after irradiation. This suggests slow nucleation of the nascent silver atoms generated within the PU film during the UVA exposure. The presence of green fluorescence (≈ 480 nm) observed in the freshly prepared films upon UVA excitation, see inset Fig. 1, agrees well with the initial formation of silver clusters that slowly nucleates to form nanoparticles.[20, 21] This emission gradually decreases with the ethanol washes, see scheme 1. Finally the polymer turned into a yellow color due to the formation of AgNP-SPB, color that is stable for at least up to two years.

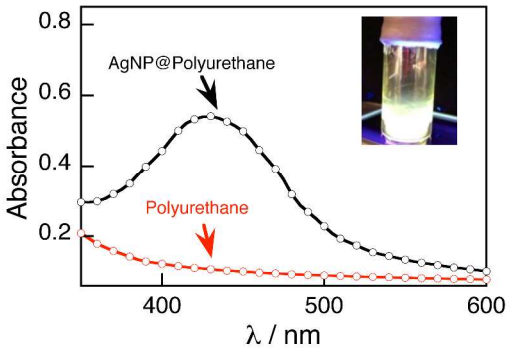

Figure 1. Representative absorption spectra for medical grade PU films (250±50 µm thickness, red circles) or AgNP@PU (black circles). Measurements were carried out at room temperature using 6 mm diameter circular pieces. Inset shows an actual picture of the green fluorescence emission observed for a freshly prepared polyurethane film containing AgNP exposed to UVA excitation.

Cryo-SEM images for the AgNP@PU films reveal the presence of silver metal nanostructures (<500 nm in average) on both surfaces (Fig. 2A) and within the material (Fig. 2B). Further, the incorporation of the nanostructures within the polymer did not produce significant changes on both T_g and FT-IR spectra of the polymers, see Table 1. This indicates minimal modifications on the supramolecular structure of polymer upon AgNP formation within the 3D matrix.

Table 1. Selected properties for AgNP@PU materials prepared in this work.

Sample code	T_g/ºC	FT-IR (cm^{-1})†
Control PU	-78±2.0	3380-3225 m (-CO-NH- and R_2-NH); 2910 and 2850 s ($R2-CH_2$); 1720 s ($R-CO-O-N-R_2$)
AgNP@PU-1	-77±1.0	3375-3220 m (-CO-NH- and R_2-NH); 2915 and 2860 s ($R2-CH_2$); 1725 s ($R-CO-O-N-R_2$)
AgNP@PU-2	-78±1.0	-

† Signal intensity strong (s) and medium (m).

Since potential biomedical uses for the materials developed here will eventually involve the interaction of the material with skin and biofluids (e.g. coating and catheters); we have assessed the impact of AgNP in the material biocompatibility using primary skin fibroblasts and human erythrocytes. Figure 3 summarizes the results for cell proliferation (Fig. 3A) and hemolysis of human erythrocytes (Fig. 3B). Those results indicate that AgNP incorporation do not impact the biocompatibility of the medical grade PU polymer. Similar results have been reported for waterborne polyurethanes containing silver nanoparticles.[15] Further, similar to the observed for skin fibroblasts, see Fig. 3A, the data obtained for the hemolysis of human erythrocytes reveal that the incorporation of AgNP reduces the basal hemolytic level of PU films ($p<0.05$), see Fig. 3B. This could be attributed to the lowering of the basal bacteria population that are adsorbed onto the material surface upon incorporation of the AgNP, see below.

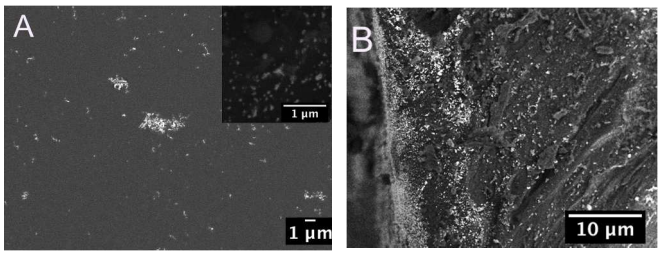

Figure 2. (A) Representative Cryo-SEM images obtained on the surface of a AgNP@PU film (15 mg Ag/g PU). Metallic silver appears as white spots. Inset shows a selected area of the polymer with a higher magnification. (B) Cryo-SEM image of a cross section of a AgNP@PU film. The left side of the image shows the top edge for the film.

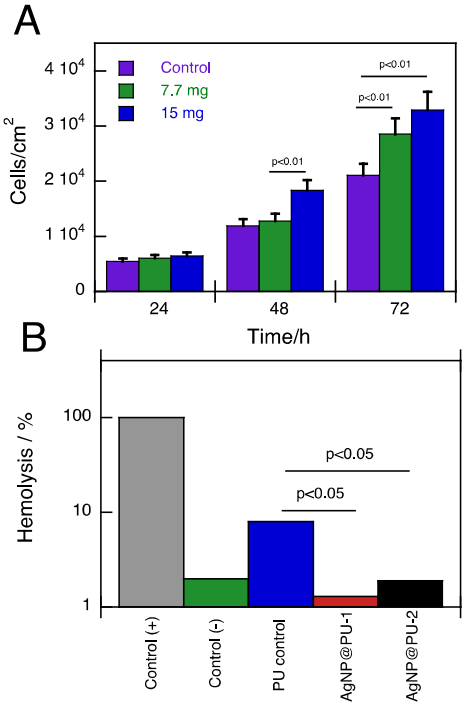

Figure 3. (A) Human skin fibroblasts cell number measured at different time intervals after cell seeding on PU films without (control) or containing two different silver concentrations (7.7 and 15 mg Ag/g PU). Experiments were carried out by quadruplicate and cell counting values measured in duplicate for each independent sample initial cell density in Fig. 3A was ≈ $2.9x10^3$ cells/cm^2.

(B) Hemolysis percentage for human erythrocytes incubated for 24 h at 25ºC in the presence of polyurethane films without AgNP (PU control) or containing 15 mg Ag/g PU (AgNP@PU-1 or AgNP@PU-2, see main text). Controls (+) and (–) correspond to experiments where the cells were completely hemolyzed using sonication and for cells incubated without any additive/polymer, respectively. *p* values shown in Figure 3A and B were calculated from Student two tails *t* statistic analyses.

As mentioned before, biofilm formation is a major problem in catheters and in biomedical devices in general. Formation of bacteria "rafts" is, among other reasons, the main cause for the development of antibiotic resistance in bacterial populations. *Pseudomonas aeruginosa*, is a Gram (–) bacteria that is directly involved in the formation and control of biofilms on skin burns and biomedical devices.[1] Notably, AgNP@PU polymers developed here are able to control the proliferation in solution of *Pseudomonas aeruginosa* (PA14) as seen in Fig. 4 top. Counting of the survival colonies after 24 h, (see Fig. 4 top) indicates that AgNP@PU have bacteriostatic behavior for PA14 proliferation under our experimental conditions. Further, experiments carried out in order to evaluate the number of colonies formed on the polyurethane surfaces for PU and AgNP@PU of PA14 biofilms indicated that the polymers containing AgNP are statistically less prone (~50%) to PA14 biofilm formation, see Fig. 4 bottom.

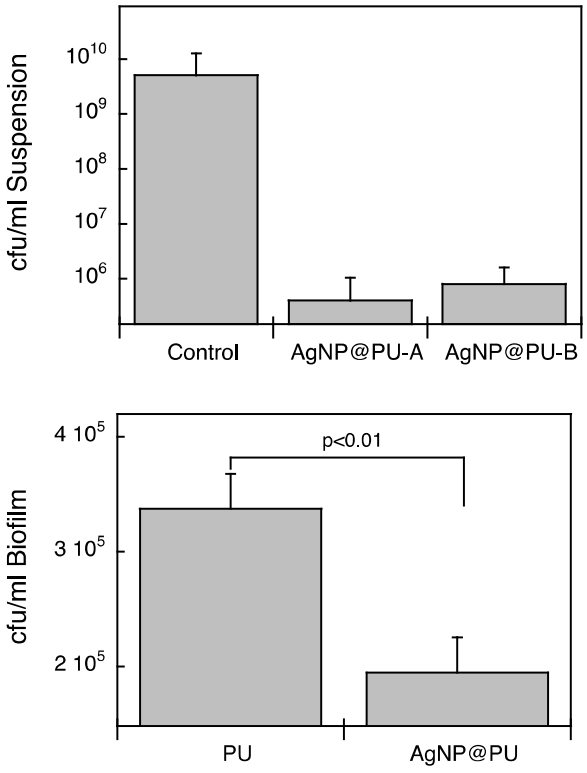

Figure 4. (Top) *Pseudomonas aeruginosa* (PA14) survival bacteria colonies sampled at 24h incubation for the control sample (PU) or for the films containing silver nanoparticles (AgNP@PU, A and B correspond to 7.7 and 15 mg Ag/g PU, UVA irradiated for 40 s, respectively) cultured in cultures at initial densities of 1x10^5 cfu/mL in enriched arginine cell culture medium (M63). Error bars correspond to the standard deviation from four independent samples. (Bottom) Colony forming units (cfu/ml) of PA14 biofilms grown on polyurethane films without or with AgNP (PU and AgNP@PU-2 respectively). PA14 biofilms were grown on 11 mm PU (n=19) and PU + AgNP (n=17) discs for 16.5 h through an ALI assay at 37˚C, see supporting information. Biofilm cells were removed from the tablets via sonication before being enumerated by a spot titer assay. After four biological replicates, the colony forming units were averaged. Error bars correspond to the standard error. The corresponding *p*-value for a two-sided Student's t-test comparing the means of the two populations is <0.01.

Conclusions

In summary, we have developed a light assisted approach for the synthesis of a composite material in which silver nanoparticles are embedded and stabilized within a polyurethane matrix. Incorporation of the nanoparticles within the material did not affect the physical properties of the material or its biocompatibility. However, the presence of silver nanoparticles in the polymer confers it antimicrobial and antibiofilm properties against the growth of *Pseudomonas aeruginosa*. Although further testing of this new hybrid polymer is needed before any translational application, the use of this material in the fabrication of catheters and/or as coating agent for medical devices would help to reduce their risk CR-BSI.

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Electronic Supplementary Information (ESI) available: Details of material preparation and characterization. Cell culture details; tests involving films. See DOI: 10.1039/c000000x/

Electronic Supplementary

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