Supporting Information

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

Sara Saez, Chiara Fasciani, Kevin G. Stamplecoskie, Luke Brian-Patrick Gagnon, Thien-Fah Mah, Maria Luisa Marin, Emilio I. Alarcon and Juan C. Scaiano

1. Chemical reagents **S2**

2. Preparation of polyurethane films with silver nanoparticles (AgNP@PU) **S2**

3. Material characterization **S2**

4. Cell culture and hemolysis experiments **S3**

5. Antimicrobial and antibiofilm assays **S3**

6. References **S4**

1. Chemical reagents

PU medical grade catheter was from Solomon Scientific, PU-C70. Silver trifluoroacetate, cyclohexylamine, THF, ethanol, and PBS were purchased from Sigma-Aldrich and used without further purification. 2-Hydroxy-1-[4-(2-hydroxyethoxy)phenyl]- 2-methyl-1-propanone (I-2959) was a generous gift from BASF.

2. Preparation of polyurethane films with silver nanoparticles (AgNP@PU)

In a typical experiment, PU sterilized catheter (350 mg) was dissolved in THF (6.0 mL). Shaking was not necessary since after 45 min the catheter was already dissolved. Then 10 mL of AgNPs precursor solution (5 mM in THF) were added.

The resulting PU solution was dried in a petri dish in darkness, under air for 24 h. The following day the film was introduced in a round bottom quartz flask with a septum, and then vacumm-N_2 steps (x3) were performed to finally leave the film under N_2 during the irradiation. Samples were irradiated in a Luzchem CCP-V4 photoreactor using 8 UVA lamps for 60 seconds. The resulting colorless film was taken from the flask and allowed for 24 h under air in the dark. After this the film was washed with ethanol for three days (3 x 50 mL) and then final washings were made using PBS for 4 days (25 mL x 3 times per day). The 5 mM AgNPs precursor solution was freshly prepared by mixing I-2959 (22.4 mg, 0.10 mmoles), CF_3COOAg (22.06 mg, 0.10 mmoles) and cyclohexylamine (114.6 μL, 1.0 mmoles) in THF (20 mL). UV-vis spectra were recorded on a Cary UV-50 spectrophotometer using 1.0 cm pathlength cuvettes.

3. Material characterization

Material characterization was carried out using low temperature scanning electron microscopy (Cryo-SEM) in a Tescan (model:Vega II - XMU) cold stage sample holder at -50ºC using a backscattered electron detector (BSE) and secondary electron detector (SED). Images were taken from the edges of the materials to further gain information regarding the distribution of AgNPs within the material. Differential scanning calorimetry measurements for PU films were carried out in a Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE) in the range of -80 to 100˚C using a scan rate of 10˚C min^{-1}. Pieces of materials between 5.0 to 10 mg in mass (Sartorius CPA225D) were hermetically sealed in an aluminum pan for analysis. The vitreous transition temperature (Tg) was measured at the onset of the endothermic peak.

In all cases the data reported corresponds to the average of at least three independent measurements.

4. Cell culture and hemolysis experiments

Human fibroblasts cells were cultured in Dulbeccos's modified Eagles's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (1.0% penicillin-streptomycin and 0.1% gentamicin) at 37ºC under 5% CO_2 and 95% humidity. PU@AgNPs films were cut in 6 mm circular pieces, and were placed into the bottom of a 96-well culture plate; 100 μL of human fibroblasts culture at 1 x 10^4 cell/mL (96% viability, counted in a Vi-Cell cell counter) were added to the wells and counted after 24, 48 and 72 h following similar protocol to the recently described by us [1]. Medium was replaced after 48 h incubation. Images were taken in an inverted microscope (Zeiss; Observer A1) using a 10X planar lens (0.013 cm_2 field view). Images were counted using ImageJ software. Control experiments were carried out using a PU film without AgNP.

A sample of freshly drawn blood from a voluntary donor was diluted 10 fold with PBS and centrifuged three times at 2500 rpm for 15 minutes. After each centrifugation, the supernatant was carefully removed and discarded, and the remaining solution, pure erythrocytes, was resuspended in PBS. This erythrocytes suspension was diluted to a final optical density between 0.4-0.8 at 650 nm (and OD=0.5 corresponds to 3.3 x 10^6 cells/ml). The hemolysis extent was determined by measuring the decreasing OD at 650 nm after 24h incubation in the presence of the polymers.

5. Antimicrobial and antibiofilm assays

Antimicrobial assays employed in this article can be classified into two main categories:

i. Evaluation of bacteria population in solution: Circular pieces (6 mm) of films with and without AgNP were cut, cleaned and placed in 96 wells. To this 100 μL of a 1x10^5 cfu/mL bacteria culture of PA14 culture in 1xM63 medium was added and culture at 37ºC in a shaking incubator. Evaluation of the survival colonies in solution after 24 h was assessed using a spot titer assay onto LB agar plates to determine the colony forming units (CFUs).

ii. Evaluation of bacteria colonies on materials surface: Biofilm formation on the material surface was evaluated for both control (PU) and PU + AgNP polyurethane materials in the form of 11 mm discs that were placed into the well of a 48-well microtiter plate. Stationary phase PA14 cultures were diluted 1:100 into 1xM63 supplemented with 0.4% arginine and 1mM $MgSO_4$ within the tablet-containing wells. The plate was placed in a 37˚C incubating chamber and tilted to an 80˚ angle to allow the biofilms to grow at the air-liquid interface (ALI). The assay was left undisturbed for 16.5 h. Following this incubation, the spent culture liquid was removed before washing each of the inoculated wells 6 times with 500 µL 0.9% NaCl. The polyurethane tablets were extracted and immersed in 500 µL of 0.9% NaCl in a 15 mL falcon tube. The immersed polyurethane tablets were vortexed for 2 seconds, sonicated for 5 minutes in a water bath sonicator, and vortexed again for 2 seconds immediately before performing a spot titer assay onto LB agar plates to determine the colony forming units (CFUs). Four biological replicates were performed.

Statistical analysis for CFU was performed using R. Statistical outliers were determined by constructing a box plot. Levene's test was chosen to assess whether the variances of the two populations were equal. The presence of a normal distribution was assessed through a QQ-plot. A two-sided Student's t-test was utilized to determine if the difference between the mean biofilm-forming CFUs of the two populations (PU and PU + AgNP) was statistically significant.

6. References

1. Vignoni, M., et al., *LL37 peptide@silver nanoparticles: Combining the best of the two worlds for skin infection control.* Nanoscale, 2014. **6**(11): p. 5725-5728.