

Supporting information to

One-step, solvent-free mechanosynthesis of silver nanoparticle-infused lignin composites for use as highly active multidrug resistant antibacterial filters

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1. Experimental

Equipment

A Retsch Mixer Mill MM 400 was used to perform milling experiments. High-resolution TEM and EDAX were performed using a Philips CM200 200 kV TEM. XPS was performed on a VG ESCALAB 3 MKII spectrometer (VG, Thermo Electron Corporation, UK) equipped with an Mg K α source. PXRD was performed using the Bruker D2 Phaser diffractometer using as CuK α source. ICP measurements were taken using a Thermo ICP-OES to measure Ag content at the elemental wavelength of 328.068nm.

Materials

Sodium (meta)periodate, ethylene glycol, and polyacrylamine solution ($M_w \sim 10\,000$, 50 wt% in H₂O) and silver nitrate were purchased from Sigma-Aldrich and used without further purification.

A Westvaco Chemical Division, Indulin AT Kraft pine lignin was purchased and used without further purification. This is a 99% lignin content free flowing brown powder lignin. 400-mesh carbon supported TEM grids were obtained from Electron Microscopy Science. Softwood thermo mechanical pulp (TMP) (FPInnovation, Pointe-Claire, Canada) were used as starting cellulose material. Cameo nylon syringe filters were purchased from Sigma-Aldrich and possess 5.0 μ m mesh size.

Periodate oxidation reaction of cellulose (Preparation of 2,3-dialdehyde cellulose)

The oxidation was carried out in aqueous media using a glass beaker with overhead stirrer, by using the following reaction conditions: Thermomechanical Pulp (TMP; 10.0 g), sodium metaperiodate (13.2 g; 61.66 mmol; 100 mole % based on moles of pulp) and sodium chloride (29.25 g; 1 N in the overall solution) were added to 500 mL deionized water. The reaction mixture was gently stirred at room temperature in the dark for 6 days. At the end of reaction, ethylene glycol was added into the reaction mixture (quenching the residual periodate) and washed with deionized water repeatedly.

Separation of lignin from oxidized cellulose

A purified oxidized TMP (2 g solid in 100 mL water) was gently stirred at 80-90°C in an oil bath for 6 hours. After this time, the sample was cooled at room temperature and non-dissolved brown solid material (mainly lignin) was separated by filtration using filter paper. The supernatant (soluble DAC and DAHC) was collected and the recovery was determined by weighing the solid material (drying at 105 °C).

Preparation of Lignin supported AgNPs

In a typical reaction, we charged a 10mL stainless steel milling jar with 2 stainless steel balls (7mm) and 0.1600g lignin and 0.0500g silver nitrate. The assembled jar was then placed into a Retsch Mixer Mill 400 and milled at 30Hz for 90min. The product was then washed with 3 aliquots of 2mL of water prior to use.

Preparation of Polymer-Lignin supported AgNPs

In a typical reaction, 1g of lignin was combined in a flask with 1mL of the purchased polyacrylamine solution which was diluted to 10mL with deionized water and sonicated for 30min. The mixture was then centrifuged for 15min at 10 000 rpm and the liquid was discarded. The solid was washed with 3, 10mL aliquot washes with deionized water. The solids were removed with centrifugation (15min at 10 000rpm) and the liquid being discarded. After drying the lignin under vacuum to dryness, 0.1600g of the polymer-lignin and 0.0500g of silver nitrate were placed into a 10mL stainless steel milling jar along with two 7mm stainless steel balls. The assembled jar was then placed into a Retsch Mixer Mill 400 and milled at 30Hz for 90min. The product was then washed with 20mL of water that was passed over the sample dropwise prior to use, using the method that is described in further detail in section 6.4.10.

ICP sample preparation

A plug was prepared by taking 50mg of the material and placing them in a plastic 5mL syringe with a syringe filter with pore sizes of 5.0 μm . The material at this point was washed with 20mL of water passed over the material using a slow drip. After which, a 2 mL sample of a water was then quickly passed through the plug using the syringe plunger. The liquid sample was then evaporated overnight in a vacuum oven and the residue was dissolved with 1mL of concentrated HNO_3 . 0.25mL of the HNO_3 solution was then diluted using deionized water up to 25mL and the sample was tested using ICP-OES at the elemental wavelength of 328.068nm.

Bacterial strains

Enterococcus faecium ATCC 19434, *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700721, and *Escherichia coli* ATCC 25922 were all purchased from Cedarlane. *E. faecium* was grown in Difco brain heart infusion media and correspondingly on brain heart infusion agar, *S. aureus*, *E. coli*, and *P. aeruginosa* were grown in Trypticase soy media and on Trypticase soy agar, and *K. pneumoniae* were grown in Nutrient media and on Nutrient agar. Difco brain heart infusion media, Trypticase soy media, Nutrient media, brain heart infusion agar, Trypticase soy agar, and Nutrient agar were purchased from BD (Becton, Dickinson and Company).

Typical organism preparation (preparation of *E. coli*)

E. coli ATCC 25922 was grown overnight in nutrient broth at 37°C. Washed cells were resuspended in water, and optical density (OD) was adjusted to under 0.1 at 600nm and compared to previously made calibration curves of the bacteria. The corresponding volume was used to create a solution of 10^8 CFU/mL of 5mL to be used in the bacterial tests.

Table S1: broth used for organism

Organism	Broth and Agar type used
<i>E. coli</i> (ATCC 25922)	Trypticase soy
<i>K. pneumoniae</i> (ATCC 700721)	Nutrient
<i>E. faecium</i> (ATCC 19434)	Brain heart infusion
<i>P. aeruginosa</i> (ATCC 27853)	Trypticase soy
MRSA (ATCC 43300)	Trypticase soy

Bacterial tests

A plug was prepared by taking 50mg of the material and placing them in a plastic 5mL syringe with a syringe filter with pore sizes of 5.0 μm . The material at this point was washed with 20mL of water passed over the material using a slow drip. A 2 mL sample of a bacterial solution containing 1×10^8 cfu was then quickly passed through the plug using the syringe plunger. The filtrate was used to make serial dilutions where 100 μL is taken and diluted with 900 μL until 8 concentrations are made. 100 μL of each dilution was taken and plated on the appropriate agar plates. The plates were then incubated overnight at 37°C. Comparison of the material's effectivity was done against control plates. Control plates were formed by passing a 2mL sample of a bacterial solution containing 1×10^8 cfu was passed through blank plugs. Blank plugs were made by taking 30mg of lignin and placing them on a glass wool plug within a pasture pipet. A 2mL sample of a bacterial solution containing 1×10^8 cfu was then passed through the plug. The filtrate was used to make serial dilutions where 100 μL is taken and diluted with 900 μL until 8 concentrations are made. 100 μL of each dilution was taken and plated on the appropriate agar plates. The plates were then incubated overnight at 37°C. For recycling test, the same operation was repeated 5 times on the same plug.

2. TEM Measurements

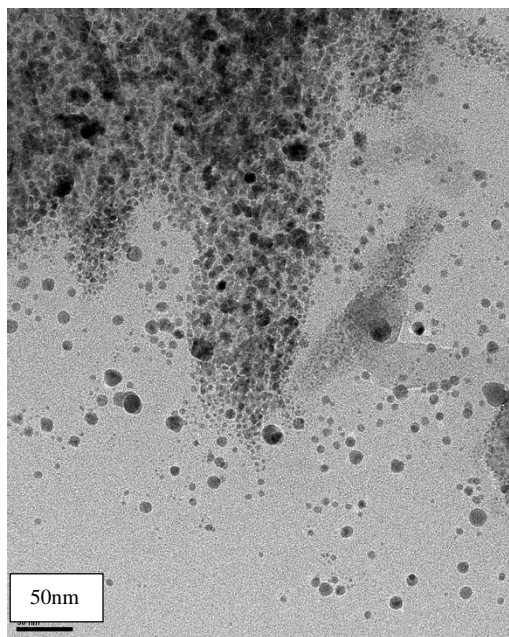


Figure S1: TEM of AgNP@Klig

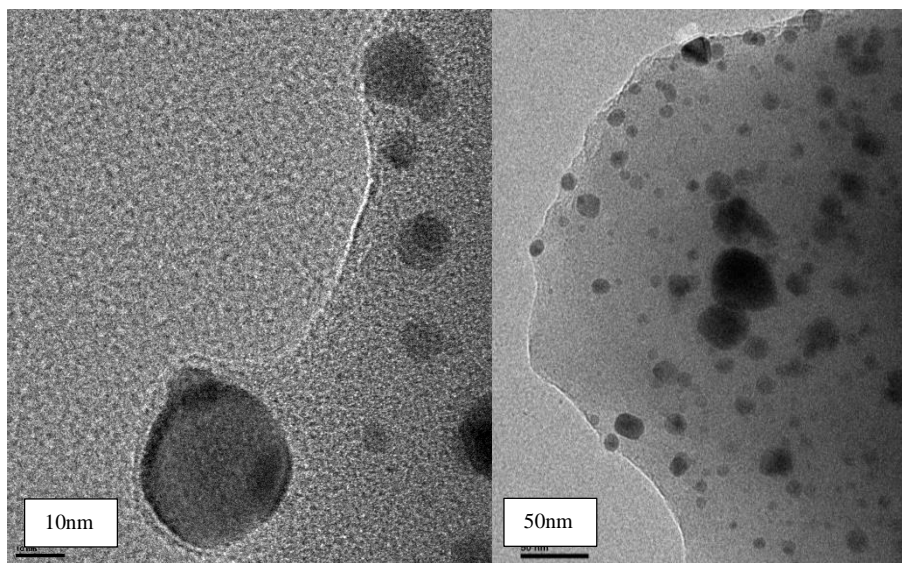


Figure S2: TEM of AgNP@Klig/PAM

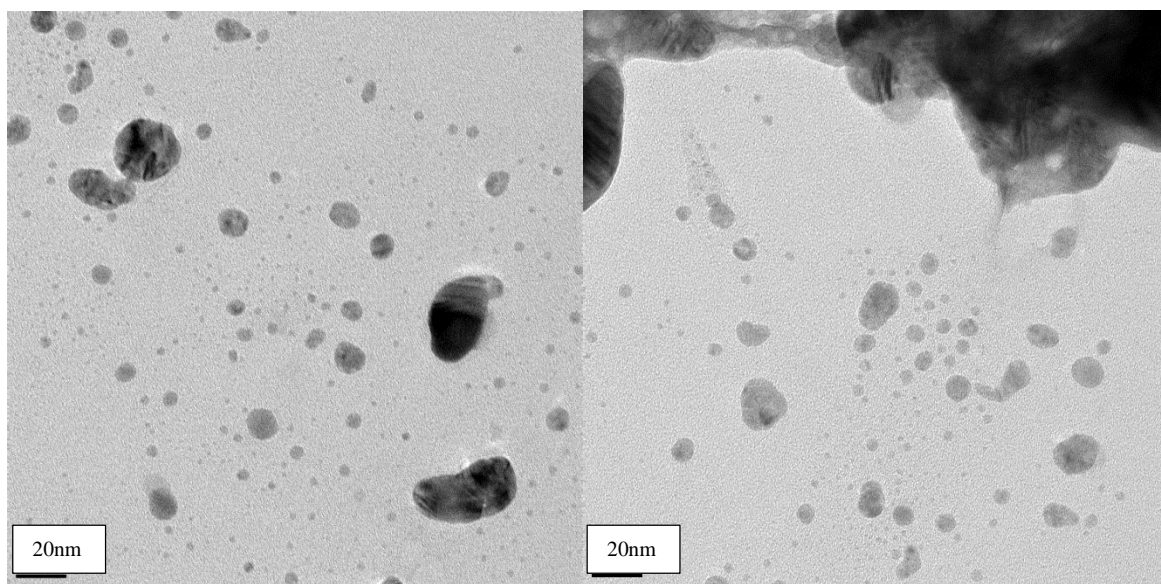


Figure S3: TEM of AgNP@TMPLig

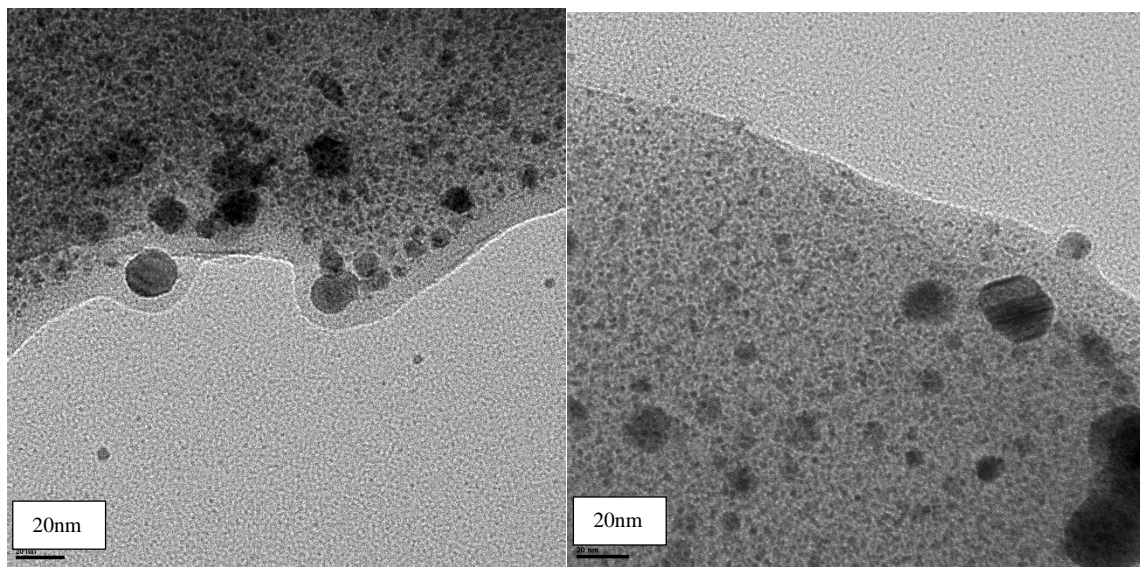


Figure S4: TEM of AgNP@TMPLig/PAM

3. FTIR measurements

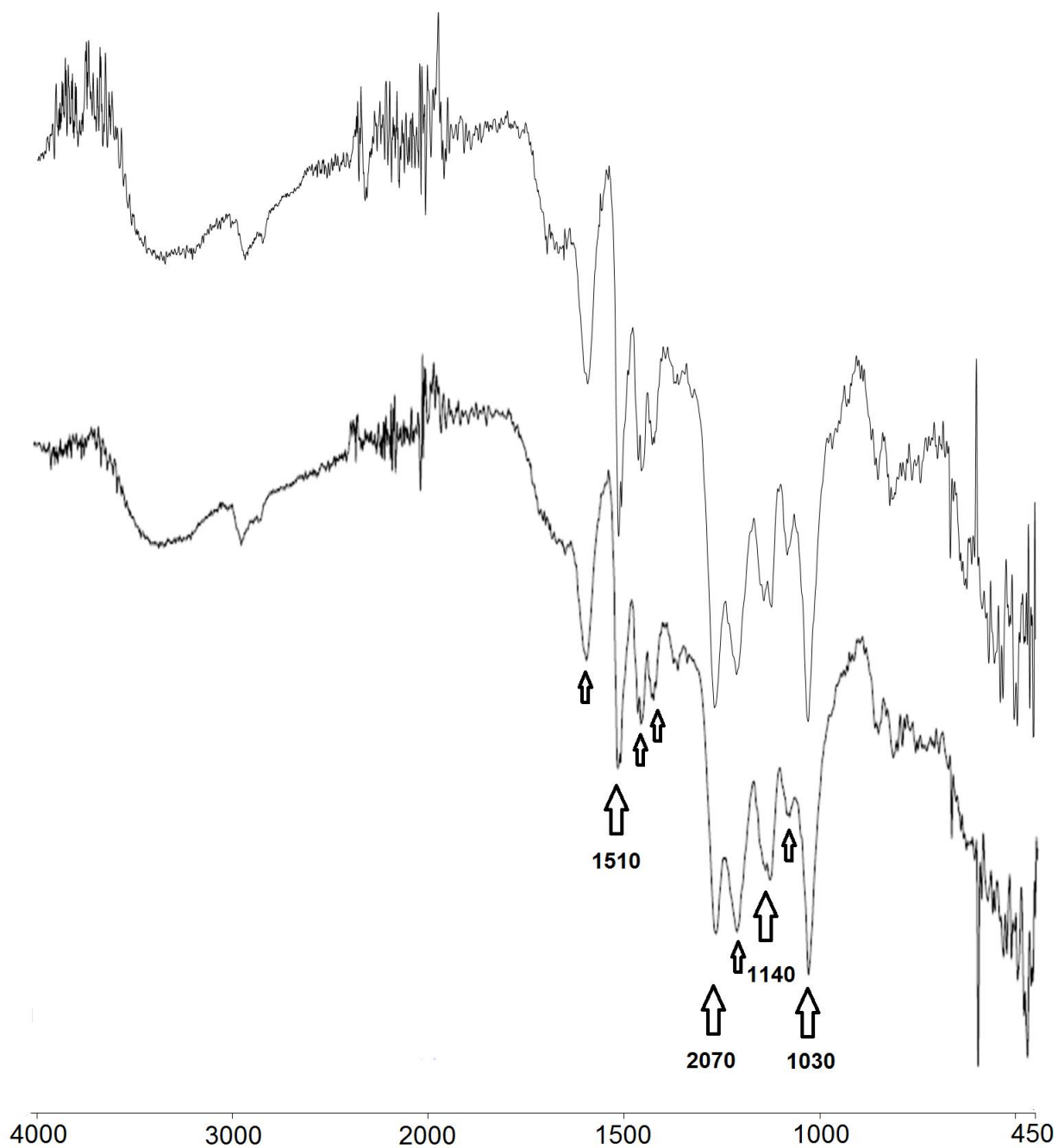


Figure S5: FT-IR spectra of Klig/PAM (top) or AgNP@Klig/PAM (bottom). Arrows point at typical peaks of lignin, the larger arrows are the most important and labelled based on O. Derkacheva, D. Sukhov, *Macromol. Symp.* **2008**, 265, 61-68.

4. XPS data

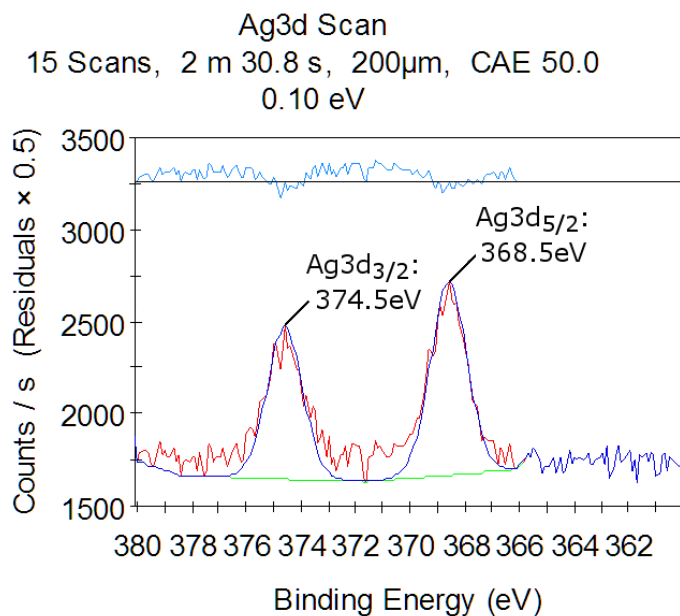


Figure S6: XPS of AgNP@KLig/PAM

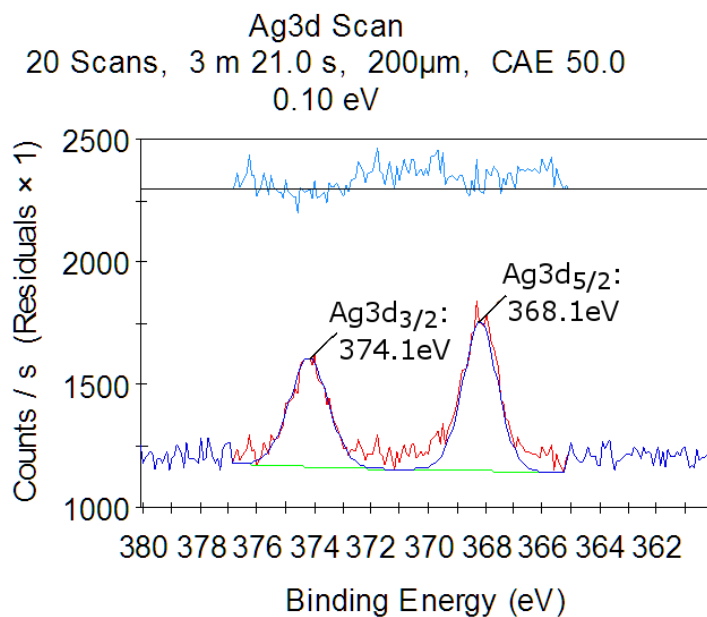


Figure S7: XPS of AgNP@TMPLig/PAM

5. PXRD data

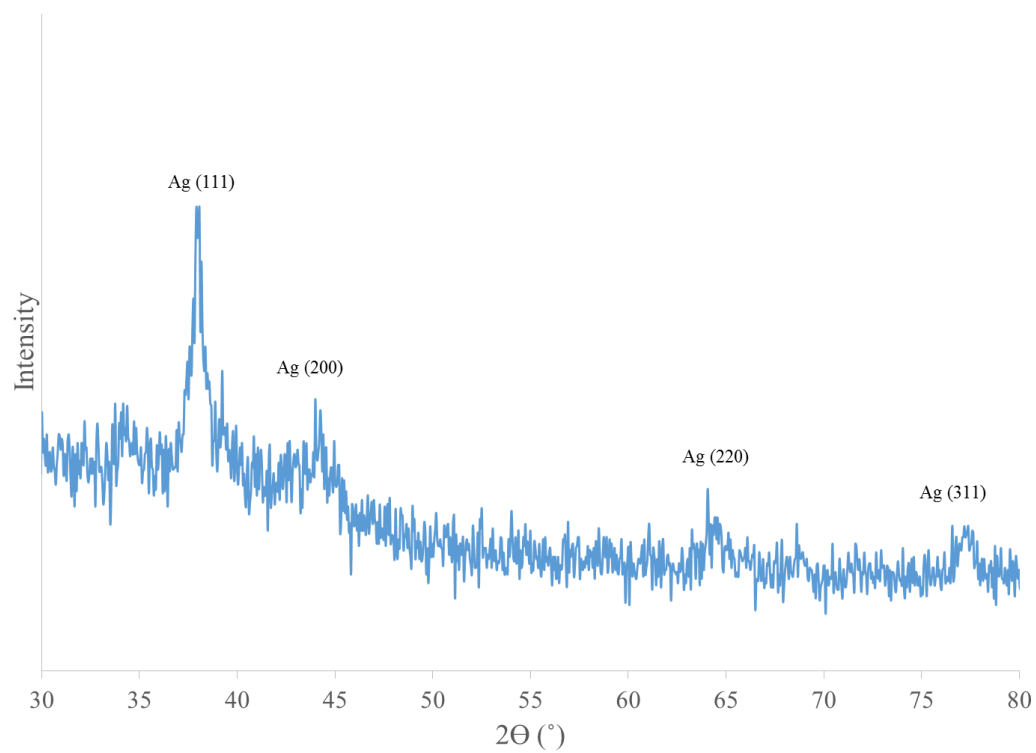


Figure S8: PXRD of AgNP@Klig/PAM

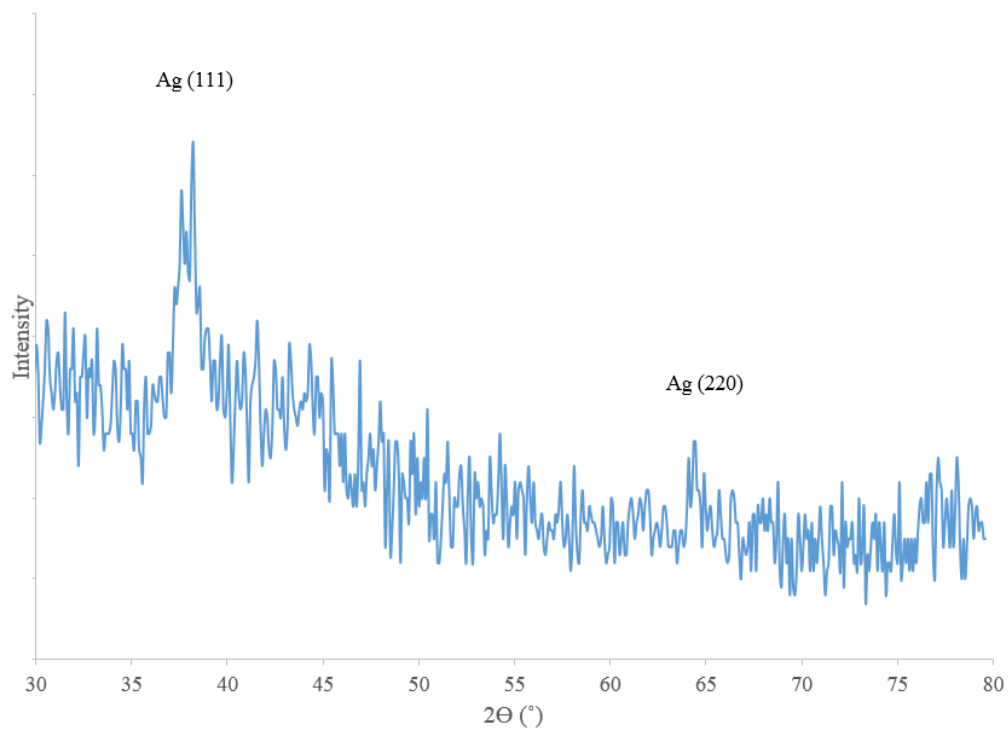


Figure S9: PXRD of AgNP@TMPLig/PAM

6. Antibacterial tests

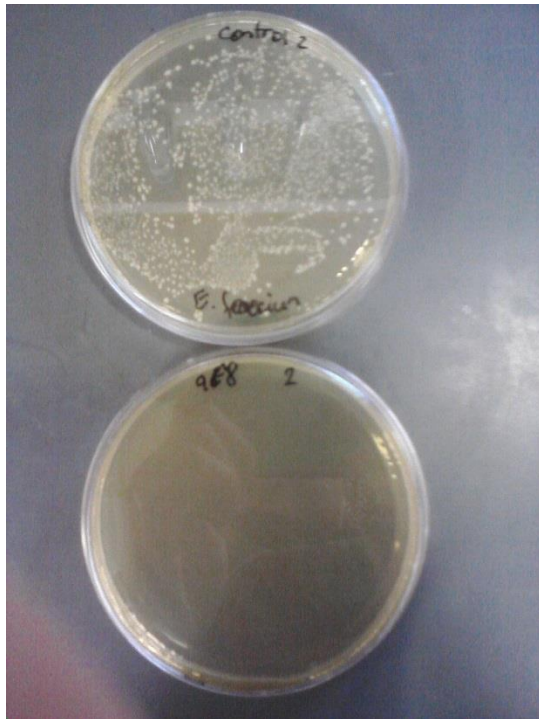


Figure S10: Photo of agar plates incubated with a solution of *Enterococcus faecium* after being passed through an AgNP@Klig/PAM equipped filter plug (top), and a Klig/PAM equipped filter plug (bottom).

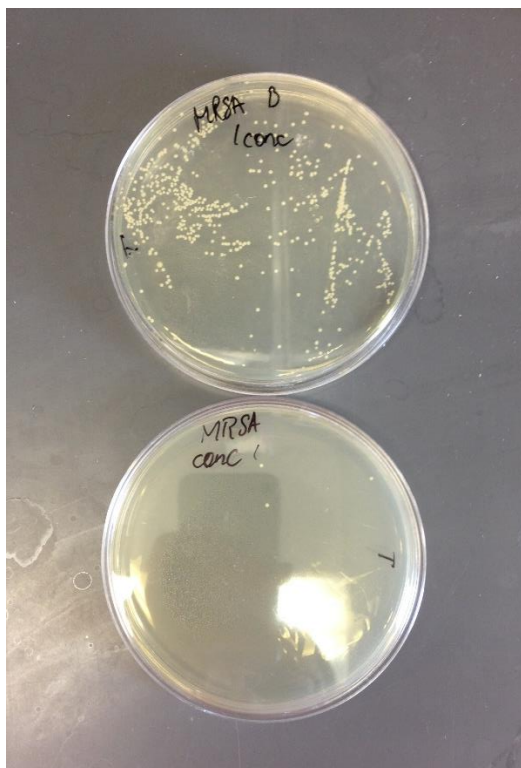


Figure S11: Photo of agar plates incubated with a solution of *Staphylococcus aureus* after being passed through an AgNP@Klig/PAM equipped filter plug (top), and a Klig/PAM equipped filter plug (bottom).

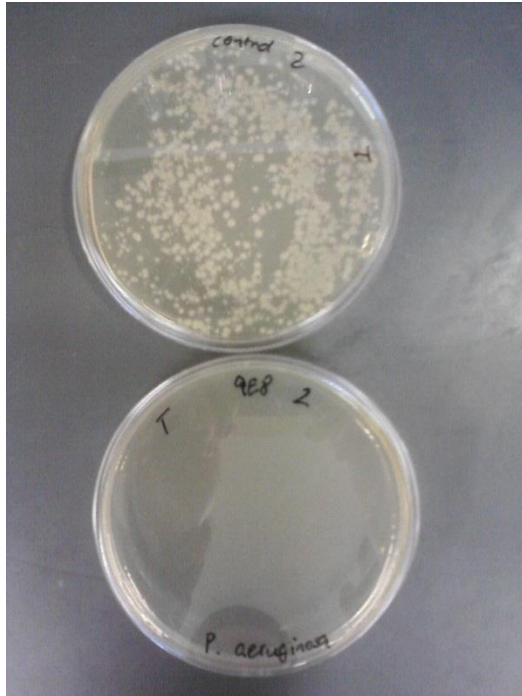


Figure S12: Photo of agar plates incubated with a solution of *Pseudomonas aeruginosa* after being passed through an AgNP@Klig/PAM equipped filter plug (top), and a Klig/PAM equipped filter plug (bottom).

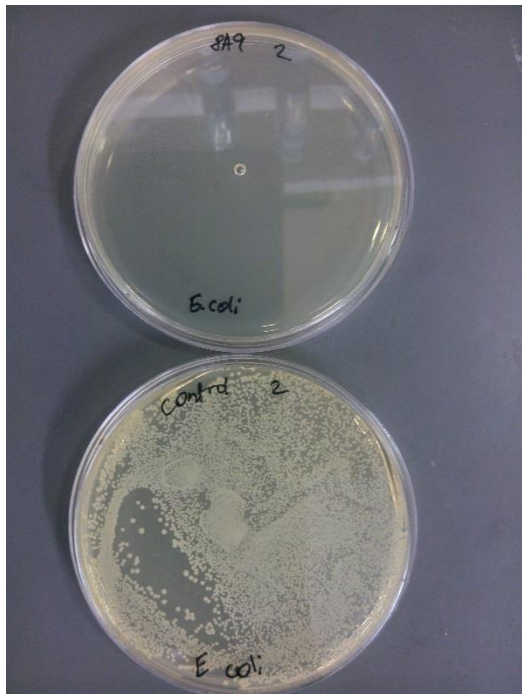


Figure S13: Photo of agar plates incubated with a solution of *Escherichia coli* after being passed through an AgNP@Klig/PAM equipped filter plug (top), and a Klig/PAM equipped filter plug (bottom).