

Supporting Information

The use of ^{111}Ag as a tool for studying biological distribution of silver-based antimicrobials

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Experimental Details

Dissolution of irradiated Palladium wire

¹⁰ Palladium wire (4 - 5.4 mg) sealed in a quartz ampoule was irradiated for one week at Missouri University Research Reactor (MURR). Separation of ^{111}Ag from the irradiated Pd target was accomplished as described by Vimalnath *et al.* with some
¹⁵ modifications.¹ Briefly, the irradiated Pd wire was dissolved in 2 mL of a conc. HCl: conc. HNO_3 (1:1) mixture by gentle heating. The resulting brownish red solution was heated gently to near dryness (it is important to avoid complete evaporation at each drying step to prevent formation of sparingly soluble oxide
²⁰ precipitates).² The solution was reconstituted in 3M HNO_3 and heated to near dryness again to expel traces of HCl. The resulting small volume was measured and an additional 2 mL of 3M HNO_3 was added. A 10 μL aliquot was removed and diluted in 1 mL 3M HNO_3 for gamma spectroscopy analysis on a high purity
²⁵ germanium (HPGe) detector (Canberra).

Separation of ^{111}Ag from Palladium isotopes

³⁰ AG1-X8 resin, 4.7 g (highly cross-linked quaternary ammonium resin attached to a styrene divinylbenzene copolymer lattice) was made into a slurry with 3M HNO_3 and loaded into the column (0.7 x 20 cm). The anion exchange resin was further conditioned with 15 mL of 3M HNO_3 . Typically, 2.64 mL of dissolved Pd wire- ^{111}Ag target in 3M HNO_3 was loaded onto the resin; the
³⁵ dissolution beaker was rinsed with additional 0.5mL 3M HNO_3 and added to the resin. The column was eluted with 3M HNO_3 and 1 mL fractions were collected for gamma spectroscopic analysis. Fraction collection was stopped when ^{109}Pd was detected in the eluted samples. Elution using 3M HNO_3 in 0.7 x
⁴⁰ 20 cm column was found to give the best separation between ^{111}Ag and ^{109}Pd . Larger weights of Pd wire were irradiated to obtain more ^{111}Ag activity increasing the consumption of nitrate ions by the Pd^{2+} ions on the resin, thus requiring more nitric acid either in concentration or amount to elute the ^{111}Ag ions.
⁴⁵ Fractions containing purified ^{111}Ag were combined and dried down under air with gentle heating in a glass vial. Samples were also analysed by ICP-MS after a suitable decay period (several months).

⁵⁰ Synthesis of $^{111}\text{Ag}[\text{SCC1}]$

The xanthinium salt, IC1 (32 mg, 0.095 mmole) was completely dissolved in 1 mL of methanol by stirring and added into a vial containing dried ^{111}Ag . This mixture was stirred for about 10 m
⁵⁵ allowing the ^{111}Ag to resuspend. For carrier added synthesis, silver acetate (29 mg, 0.174 mmole) was added into the ^{111}Ag -IC1 solution. A yellowish solution developed, which turned dirty yellow after 10 m of mixing; an additional 300 μL methanol was added to improve solubilisation. The final mixture was stirred at
⁶⁰ room temperature for 2 h. After 2 h, the residue was centrifuged at 2500xg for 5 m. The supernatant was collected and the desired product isolated by precipitation with 35 mL of cold diethyl ether (previously kept in dry ice for 1 h) in a 50 mL conical tube. The product was collected by centrifugation and dried under air with
⁶⁵ gentle heating. Chemical yield was estimated from the weight of the product of a cold reaction perform in parallel with the radioactive reaction.

Biodistribution Studies

⁷⁰ All animal experiments described were performed according to animal use protocols approved by the Washington University Institutional Animal Care and Use Committee (IACUC). *In vivo* studies were conducted by administration of aerosol doses to
⁷⁵ C57BL/6 mice via direct nebulization of the silver carbene complex, [^{111}Ag]SCC1 into the lungs of normal mice in a multi-dosing animal chamber as shown in figure S3 below. The multi-dosing chamber used was a square Plexiglas box with inner dimensions of 8 L x 8 W x 4.5 H inches with a nebulizer mounted
⁸⁰ in the center of the lid. Animals were individually placed into Plexiglas tubes designed to hold 4 mice individually for nose-only delivery. The nebulizer, obtained from Aerogen, has a micropump technology that produces particles (4-6 μm) in a low velocity aerosol. Two types of aerosol delivery experiments were
⁸⁵ carried out; one dose administered once for 5 m or five doses administered in a span of 3 days. The mice were kept in metabolism cages overnight for the 24 h biodistribution when mice were sacrificed and organs of interest collected and measured for radioactivity content. The administered dose was
⁹⁰ 200 μL (16-40 μCi)/ 4 mice administration.

Supporting Information

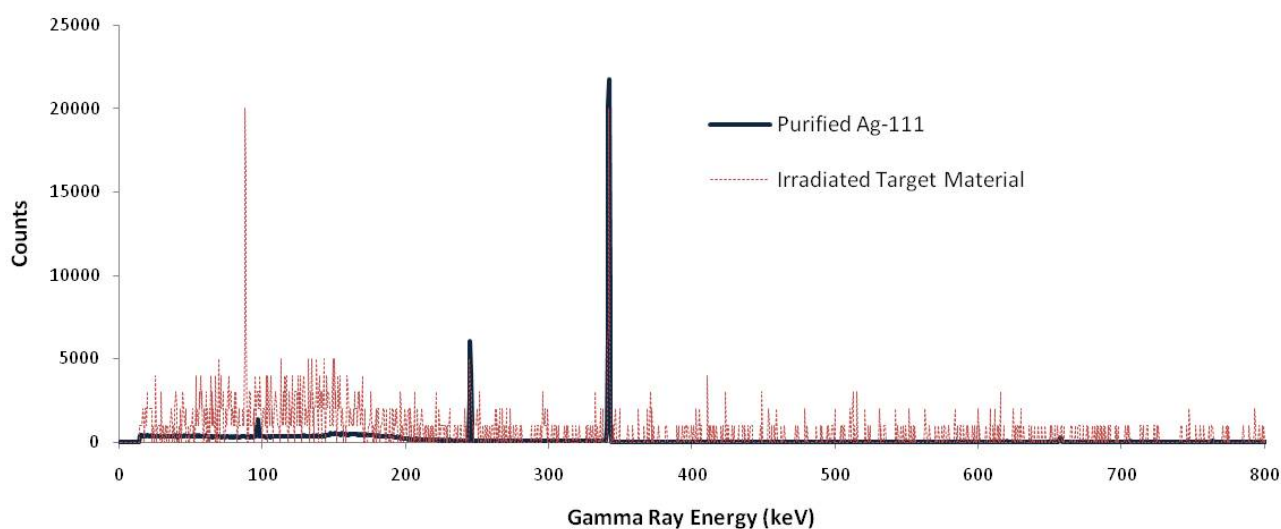


Fig. S1 Gamma Spectra of irradiated Pd target overlaid on the purified ^{111}Ag sample. Characteristic gamma ray energy (keV) of purified ^{111}Ag is shown as the bold black lines at 96.8 (0.11%), 242.2 (1.33%) and 342.1 (6.68%). The characteristic gamma energy for ^{109}Pd is depicted as the dashed red line at 88 keV in the crude irradiated target.

5

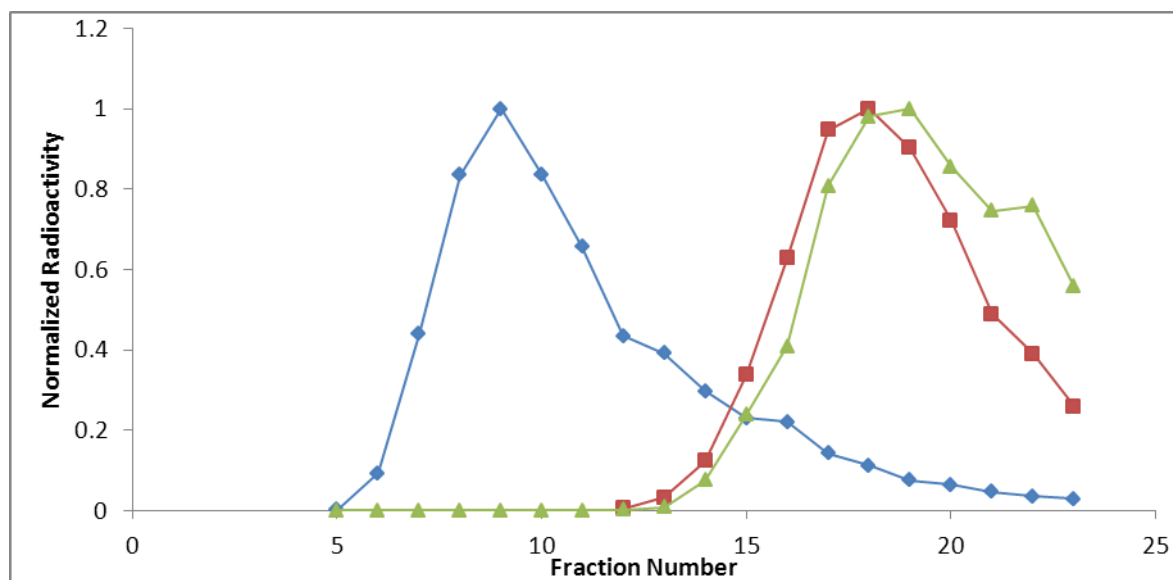


Fig. S2 Chromatograph showing the elution profile of ^{111}Ag from AG1-X8 anion exchange resin. A sharper, faster elution profile of ^{111}Ag was observed when 3M HNO_3 was used (blue line) on a 0.7 x 20cm column, while ^{111}Ag was slowly eluted from a column with dimension 1.0 x 10cm (green line) with 3M HNO_3 . A similar slow elution profile of ^{111}Ag was observed with 2M HNO_3 on a 0.7 x 20cm column (red line).

10

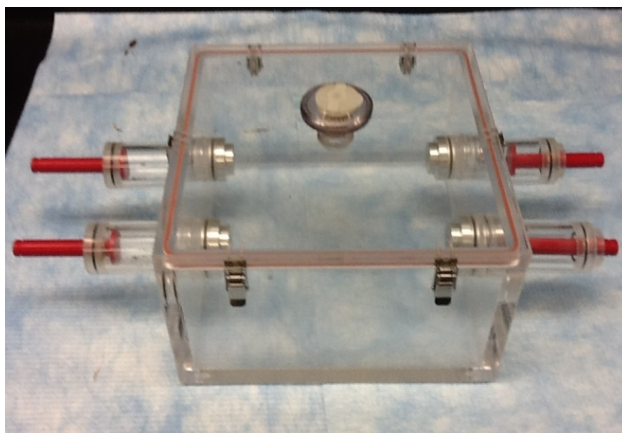


Fig. S3 Multi-dosing animal chamber designed to hold 4 mice individually in the Plexiglas tubes for nose-only delivery.

References

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