Electronic Supplementary Information (ESI)

Plasma Deposited Silver Containing Antimicrobial Films

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Synthesis of complex **1**.

To a stirred solution of maleimide (10mmol, 0.98g) in ethanol (30ml), 10ml of an methanolic solution of silver nitrate (10mmol, 1.7g) was added and the solution stirred for 5 minutes. After this time a solution of triethylamine (10mmol, 1.01g) in ethanol (5ml) was added dropwise over 1 hour, resulting in the formation of an insoluble white precipitate. The solid residue was isolated by filtration, washed three times with ethanol and dried *in-vacuo*, yielding silver maleimide (1.92g, yield 94%). Addition of silver maleimide (5mmol, 1.02g) to a stirred solution of triphenylphosphine (10mmol, 2.62g) in toluene resulted in the formation of an off-white precipitate after 1 hour. The solid residue was isolated by filtration, washed three times with toluene and dried *in-vacuo*, yielding *bis*-triphenylphoshino silver maleimide (**1**) (3.35g, 92%)

Recrystalisation from *iso*propanol resulted in the formation of crystals suitable for single crystal X-ray diffraction studies. Anal. Calc. for : C, 65.48; H, 5.12; N,1.78: Found: C,64.7; H,5.40; N,1.38; ^{1}H NMR (300 MHz, 23°C), $CDCl_3$ (ppm): $\delta =$ 7.3 (m, 30H, P(C_6H_5)$_3$.), 1.9 (s, 2H, -C*H*-C*H*-) ; ^{13}C{^{1}H} NMR (75 MHz) $\delta =$38.98 (s), 129.28 (d, J=10 Hz), 130.49 (d J=2 Hz), 133.01 (d J= 25 Hz), 134.41 (d, J= 16 Hz) ppm; ^{31}P {^{1}H}NMR (121 MHz) (ppm) $\delta =$ 10.00 (s, *P*Ph$_3$).

FTIR spectroscopy shows disappearance NH stretch in (**1**) compared to the free maleimide and IR absorptions at: 3132 cm $^{-1}$, 2967 cm^{-1} (C-H, Ph alkene), 1616 cm^{-1} (C=O), 1478 cm^{-1}, 1433 cm^{-1}(ring deformation), 1327 cm $^{-1}$, 1281cm^{-1}, 1249 cm^{-1}(conjugation), 1155 cm^{-1}, 997 cm^{-1}and 694 cm^{-1} (P-C). The carbonyl peak in

PSSM is also significantly different that observed for the free maleimide at ~1615cm^{-1}. This change can be ascribed to the charge delocalization about the maleimide ring and a reduction of the C=O bond order. A shoulder peak attributed to the alkene stretch is also seen at around 1600 cm^{-1}. Consistent in both FTIR spectra are peaks between 1240-1360 cm^{-1}, ascribed to the C=C bond in the maleimide ring. In addition, carbon-hydrogen bending modes associated with *cis*-alkenes appear at around 940 cm^{-1} for both compounds. Peaks seen in maleimide for interactions between C-N stretching and N-H bends are not present in PSSM spectra, while a multitude of additional peaks diagnostic of triphenylphosphine in the fingerprint region are shown, allowing for extra confirmation of the new molecule. These absorptions are also not seen for pure silver maleimide.

X-ray Crystallography

The Crystallographic data for PSSM is summarised in Table 1. Data collection was implemented on a Nonius Kappa CCD diffractometer, using Mo-Kα radiation (λ = 0.71073 Å). Data were corrected for Lorentz and polarization effects, and structure refinement was by full-matrix least squares on F^2. Structure solution and refinement was performed using SHELX-86[1] and SHELXL-97[2] software, respectively. Data were uniformly corrected for Lorentz and polarisation. Hydrogen atoms were included at calculated positions throughout, and refined using a riding model.

In the crystal structure of (**1**) in addition to a molecule of the complex, the asymmetric unit also contains a molecule of *iso*-propanol and one molecule of methanol, disordered over two positions. Maximum convergence was attained by treating this latter volume of the electron density map with a free variable, which was subsequently refined to 50% occupancy on both sites and refined isotropically. While CH hydrogen atoms are included in the refinement in calculated positions, only the Hydrogen of the *iso*-propoanol unit (OH) was freely found in the difference map. The hydrogen atom belonging to the disordered methanol unit (OH) was not freely found, but is included in the unit cell contents.

Table 1 Crystal data and structure refinement for (**1**)

Formula	$C_{44}H_{43}AgNO_4P_2$
Formula weight	820.61
T / K	150(2)
Crystal system	Triclinic
Space group	*P-1*
a / Å	12.2293(3)
b / Å	13.2667(3)
c / Å	13.3833(4)
α / °	91.7750910)
β / °	97.0150(10)
γ / °	115.2380(10)
/ Å^3	1941.01(9)
Z	2
D_c / g cm^{-3}	1.404
μ / mm^{-1}	0.645
F(000)	846
crystal size / mm	0.50 x 0.25 x 0.18
Theta range / °	3.99 to 30.04
Reflections collected	32127
Independent reflections	11156 [R(int) = 0.0491]
Reflections [$I>2\sigma(I)$]	8838

X-ray crystallography also shows the presence of both *iso*-propanol and methanol of crystallisation in the product, an observation that is confirmed by both NMR spectroscopy and elemental analysis.

MIC determination

An initial 6 solution dilution series of compound **1** was made up in molecular biology grade DMSO (>99.99% purity) with a range of 4.8 mmol dm^{-3} to 0.48 nmol dm^{-3}. DMSO was used due to the insoluble nature of the two monomers and must be >99.99% pure to avoid contaminants such as iron, that are present in >98% pure DMSO. Once this initial dilution series is made up, 100µl of each solution was added to 800µl LB and 100µl 10^6 CFU/ml PA01 in LB. The samples were incubated at 37^{o}C for 18 hours, after which they are removed from incubation and the bacteria concentration determined by plating out on agar and colony counting.The results of this assay provide an estimate for the minimum concentration required to inhibit bacterial growth.

Zone of inhibition for *P. aeruginosa*

Pellets of compound **1** were prepared using a 13 mm diameter dye and 10 tonne press. Required molar quantity of compound **1** and KBr were weighed, added to the die and pressed at 8tonnes for 30s. Overnight cultures of PA01 and MSSA in LB media were adjusted to 10^5 CFU/ml. 100µl of solution was spread onto a 2.5% LB agar plate, and a pellet placed onto the dish immediately. Plates were inverted and incubated at 37ºC for 18-24h. After this time, zone of inhibition was measured as distance between edge of pellet and edge of bacterial growth ring.

Petri dish assay

Plasma deposited compound **1** was deposited onto three 50mm diameter Petri dishes in three separate depositions (1h, 50W, CW), post 10min O_2 50W CW plasma. (NB: no pp-C_2F_6 stage) Control Petri dishes were subjected to 10min UV/O_3 treatment in order to sterilise.

Overnight cultures of *P. aerginosa* were adjusted to ca.10^5 CFU/ml in minimal LB media (10x dilution of regular LB), 40µl of which was added to each Petri dish with sufficient minimal LB to make a working solution of 4ml at 10^3 CFU/ml. Dishes were incubated at 37ºC for 18-24h. After this time, effluent media was poured off and surface indirectly washed with 2x 2ml physiological saline (0.9% *w/v* NaCl). 1ml of physiological saline was applied to dish surface, before each dish was wrapped in parafilm and vigorously shaken for 30s. 100µl of the vortexed solution was applied to

the surface of a 2.5% LB agar plate and spread. Further vortexed solutions at 20-, 400-, 8000-, 1.6x10^5-, and 3.2x10^6-times dilution in physiological saline were also made and spread in the same manner. Plates were incubated for 18-24h until bacterial colonies were observed.

Japanese industrial standard (JIS) assay

Plasma deposited compound **1** was deposited onto three 9x9cm strips of non-woven (PP) in one deposition (3h 100W, CW), followed by a subsequent adlayer of pp-C_2F_6 (1min, 50W, CW). a These strips were cut into three sets of two 3x3cm squares. Control non-woven were cut into 3x3cm squares and subjected to 10min UV/O_3 treatment in order to sterilise.

Overnight cultures of PA01 and MSSA were adjusted to ca.10^5 CFU/ml in LB media, 200µl of which was added to each square of non-woven in a sterile 50mm petri dish. Infected non-woven was incubated at 37ºC for a specified time. After this time, non-woven squares were removed from the Petri dish, lightly washed in a 4ml physiological saline (0.9% *w/v* NaCl) dip and placed into a 50ml tube containing 20ml of physiological saline. Each tube was then vigorously shaken for 5 x 5s. 100µl of the vortexed solution was applied to the surface of a 2.5% LB agar plate and spread. Further vortexed solutions at 20-, 400-, 8000-, 1.6x10^5-, and 3.2x10^6-times dilution in physiological saline were also made and spread in the same manner. Plates were incubated for 18-24h until bacterial colonies were observed.

Colony counting

After incubation, agar plates containing colonies of bacteria were assessed for viable cell count. A dilution factor was chosen where individual colonies were evident, but at countable and statistically viable levels (ca.30-300). Colonies were then counted and scaled up (multiplied by 10x dilution factor) to give an estimation of viable cell count, colony-forming units per millilitre (CFU/ml).

Plasma deposition system

A reactor comprising of a 30cm-long, 10cm diameter Pyrex tube and stainless steel discs at each end, both connected to ground, attached to an Edwards RV5 pump

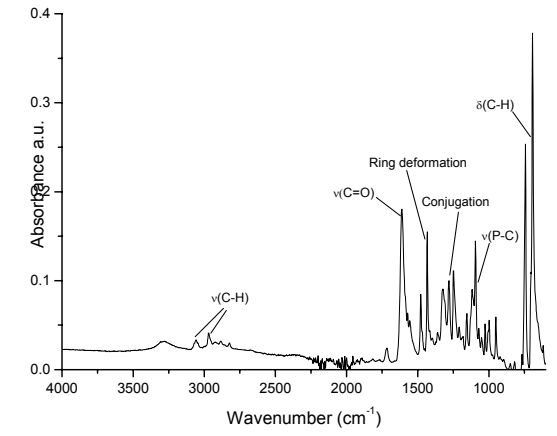
(used to create a vacuum of ca. 10^{-2} mbar) was used for these experiments based on the design described by Bullett et al. [3] Oxygen for reactor cleaning was fed in via a gas control valve and monomer vapour introduced via a Young's tap. A 13.56 MHz RF coaxial system supplied power and was connected to the reactor via a 2mm-diameter coil of copper wire, earthed through the electrodes. A manual matching unit was used to adjust input impedance, ensuring minimal reflected power (>0.5%) and an oscilloscope allowed pulsing of the input power.

Plasma deposition parameters are given in table 1:

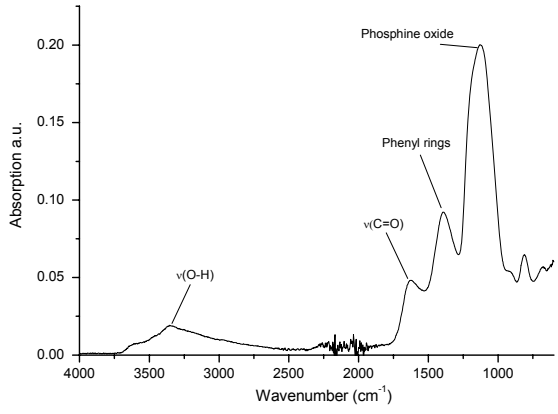
Duty cycle (peak power)	Flow rate / sscm^3 min^{-1}	Base pressure / mbar	Film deposition rate	Deposition time
CW (50 W)	8+/- 2	0.012	0.2 nm min^{-1}	1h – 3h

Table 1: Plasma deposition parameters for compound 1.

FTIR data for polymer



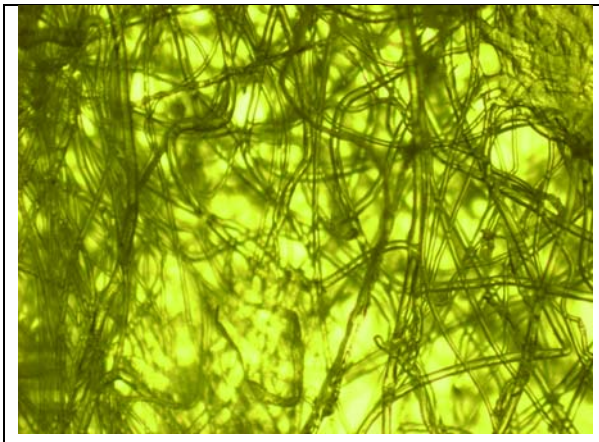
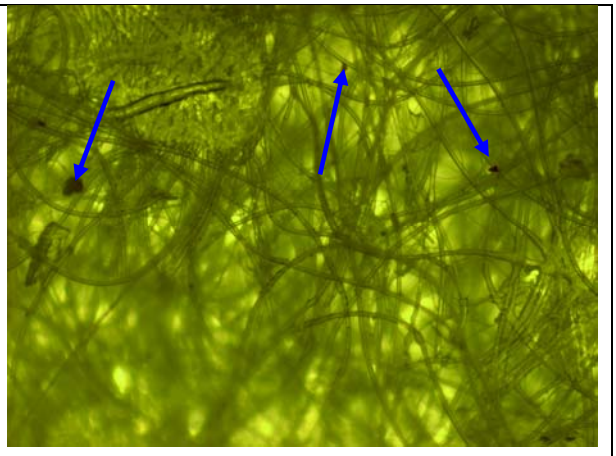
ATR-FTIR of recrystallized PSSM monomer



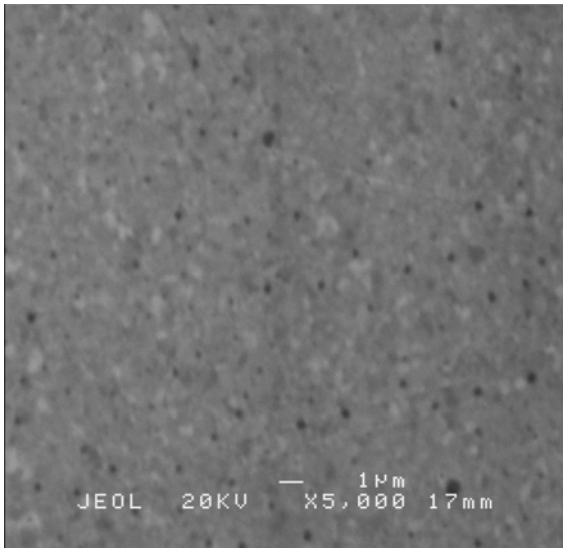
ATR-FTIR spectra of thin film (ca. 50 nm) of PSSM deposited under continuous wave, 50W conditions. Note evidence of OH or NH stretch at 3400 cm-1, previously absent, and general broadness of peaks typical for polymer spectra.

Microscopy of non-woven fabric prior to, and following deposition

Light microscopy showed that no melting or damage to the non-woven material took place during deposition, but dark areas appeared on the fibres, which are attributed to deposited silver.

	
Microscope image of non-woven prior to deposition Fibre diameters ca. 10 μm	Microscope image of non-woven following plasma deposition of compound 1. Note dark material (shown by blue arrows)

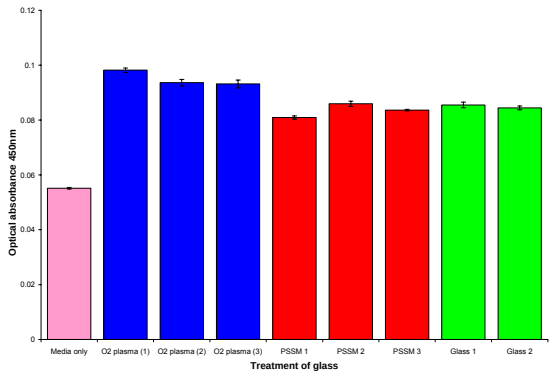
Scanning Electron Microscopy of the plasma deposited compound 1 on gold substrates also showed a pattern of dark patches, smaller than seen in the light microscopy on the fabric. EDX analysis showed these patches to contain silver.



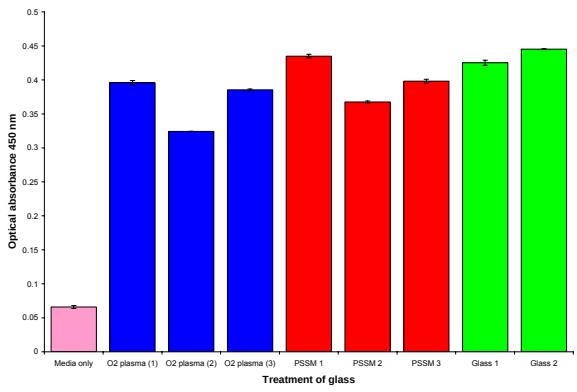
Scanning Electron Microscopy image of plasma deposited compound 1 on planar gold.

Cytotoxicity

The cytotoxicity of PSSM was studied by culturing cells from embryonic Swiss mouse fibroblasts and human neonatal epidermal keratinocytes glass coated with PSSM, oxygen plasma cleaned glass and untreated glass. Mouse cells grown for 24h, neonatal human cells for 72 h. Cell viability was measured using an XTT / MPMS assay, where the XTT tetrazolium is converted to soluble orange formazan crystals by mitochondrial enzymes in living cells, hence giving a colourimetric response, measured by visible light absorption at 450 nm.[4] The higher the concentration of orange formazan, the greater the number of healthier (metabolically active) cells.



Human epidermal keratinocytes



Swiss mouse fibroblasts

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Electronic Supplementary Information (ESI)