Supporting Information

Transparent Anti-SARS-CoV-2 and Antibacterial Silver Oxide Coatings

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Additional Methods:

Antibacterial Assay (Continued)

Confirmation of Surface Killing. It is in principle possible that the low counts that were observed on antimicrobial coating were due to a poor yield in rinsing bacteria off the coating prior to the assay. To rule out this possibility, we assayed *P. aeruginosa* and MRSA that remained adherent to the coupons after vortexing and sonication. These coupons were placed with the coating in contact with a TSA plate. After 10 min, the coupons were removed and plates incubated at 37° C to determine whether any adherent, viable cells were present on the coupons and able to form colonies. For the experiments reported here, no colonies appeared indicating that no viable cells remained adhering to the coupons.

Surface Killing Not Influenced by Post Exposure to Particulate Silver. It was possible that particulate silver oxide was released during the vortexing and sonication and could kill suspended cells during the assay, thereby giving the appearance that bacteria were killed on the coating. To rule out this possibility, in separate experiments, coupons were suspended in DE-buffer (BD, Sparks, MD) as well as PBS. DE buffer is composed of agents able to block or inhibit killing by a wide variety of antimicrobial agents, including metals.[1] For the experiments reported in this paper, there was no reduction in killing *P. aeruginosa* and MRSA when coupons were vortexed and sonicated in DE buffer, compared to PBS. Thus, there was no killing of suspended cells by silver oxide released from coupons.

EPA Abrasion Test

Prior to distribution of antimicrobial coatings in the United States (US), the US Environmental Protection Agency (EPA) requires testing of the antimicrobial efficacy after abrasion. We carried out a variant of EPA-approved test to assess whether the coating was sufficiently durable. In this method, a sponge is repeatedly translated across a surface by a Gardco, Model D10V abrasion 214 tester, and then the antimicrobial activity of the abraded surface is assessed. The sponge (Brite Non-Scratch Scrub Sponge, model C05068) is initially autoclaved and left in laminar flow hood to completely dry overnight. Next, the sponge is placed in 20 mL of disinfectant for ten minutes and then the partially wet sponge is attached to the boat of the machine and used to simulate exaggerated wear on coatings. We modified the EPA test by using 70% ethanol instead of their disinfectants because our application is electronic displays and most people use alcohol to clean electronic displays. The tester translates the sponge parallel in contact with the active surface of the sample with a load of 0.45 kg, an amplitude of ~26 cm, and a period of 2.2s/pass. The sponge was wetted with 20 mL of 70% ethanol in water solution prior to the abrasion tests. A single cycle consists of the translating the sponge across the sample 8 times, then 70% ethanol is sprayed on the abrasion platform, and the test coating, and then there is a subsequent 30 minutes waiting period. Ten cycles were performed for a total of 80 passes. A single sponge was used for the first 5 cycles and this was replaced with a second sponge for the second 5 cycles.

Repeated Exposure of Bacteria to Coating

In order to determine the efficacy of this coating after multiple exposure to microbes, a series of exposure/bacterial assay/cleaning steps were carried out on the coating. First, a 10 µm droplet contaning 2.6×10^5 CFU of *P. aeruginosa* was placed for on the coating. After one hour, the coating was put in 5 mL DI water, vortexed for 1 min and then sonicated for 1 min and the eluant was used in CFU measurements. Next, the previously used samples were recovered and cleaned. Cleaning constisted of soaking in 3% Lysol® for for 30 min followed by 3x rinsing with 70% ethanol in water and 3x rinsing with DI water. Samples were then dried for 15 minutes before the next exposure. Figure S16 shows the antibacterial activity of this coating after 4 exposure cycles.

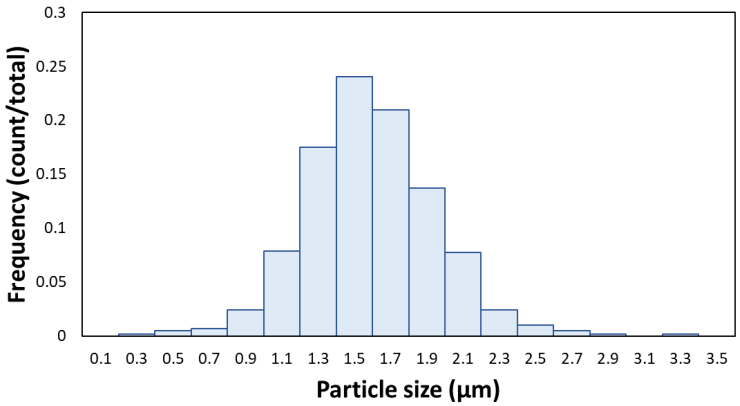

Figure S1 Ag_2O particle size distribution. Results show average and median size of 1.5 µm. Over 300 particles were included in size analysis and SEM images were analyzed using ImageJ.

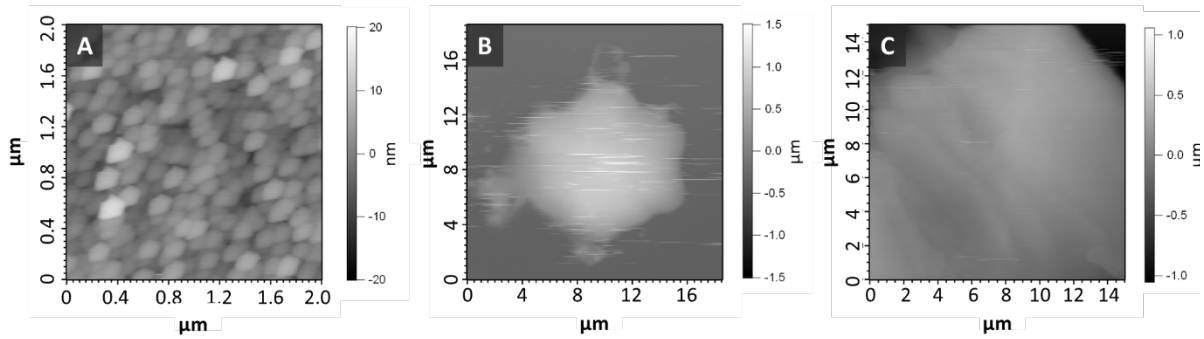

Figure S2 AFM images showing the height of the particles in the coating. A) Region where particles are not present, B) region with particle, and C) the surface of a particle in the coating. Note that large change in vertical scale between parts A and B.

Table S1 Surface elemental analysis of coating by EDS. Two regions of Ag_2O-coating were tested, we expect an Ag:O ratio of 2:1 whereas we observe 2.5: 1.

		%Ag	%O	Ag/0
Region 1		68.7	31.3	2.2
Region 2		73.7	26.3	2.8
Mean value%		71.2	28.8	2.5

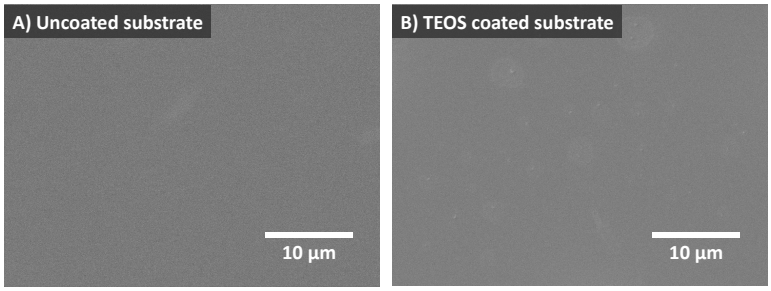

Figure S3 SEM images of A) uncoated substrate and B) TEOS coated substrate. Without the Ag_2O particles, the surface is very smooth and shows no features in SEM.

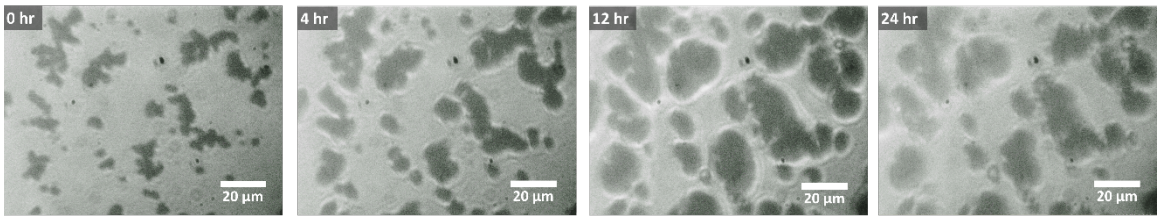

Figure S4 Time lapse of sol-gel reaction: During the sol-gel reaction, particles will be partially dissolve and self-assemble to form the final morphology of particles in the coating.

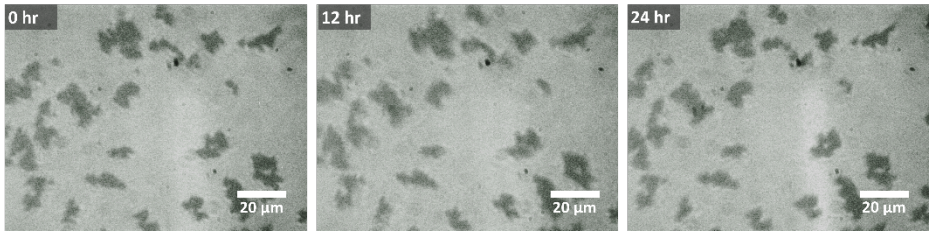

Figure S5 The effect of ammonia on the morphology of silver oxide particles. These light microscope images show the time course of film formation when no ammonia is present. By comparison to Figure S3, it is clear that the Ag_2O particles do not change morphology, so the change is caused by ammonia. Note that ammonia is necessary for the sol-gel reaction, so the coatings shown in this figure are not robust because silica does not form.

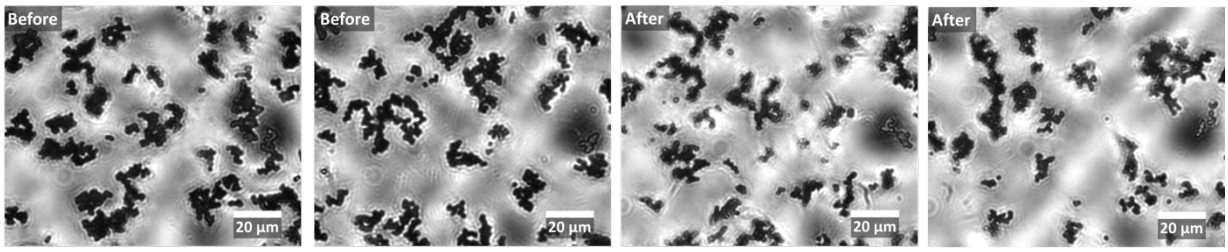

Figure S6 The effect of heat treatment on the morphology of silver oxide particles. Comparison of the surface before and after one hour of heat treatment at 50 °C shows no change on a 1-20 µm scale. Images were captured using a Zeiss Axio Imager M2 microscope transmission mode.

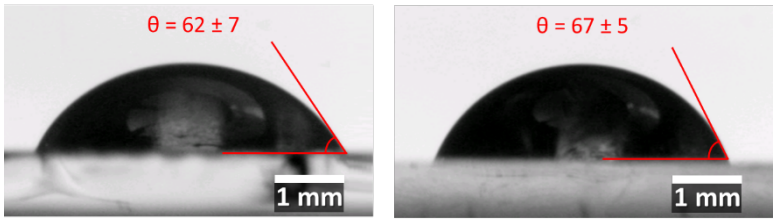

Figure S7 Photographs of 10 µL water droplets on the coatings. Numbers in red indicate the 95% confidence interval of static contact angle values for Ag_2O and $2xAg_2O$ coatings. The 95% confidence interval of advancing and receding contact angle values are 71.5±5 and 31±9 for Ag_2O-coating and 75±1 and 35±4 for $2xAg_2O$-coating respectively.

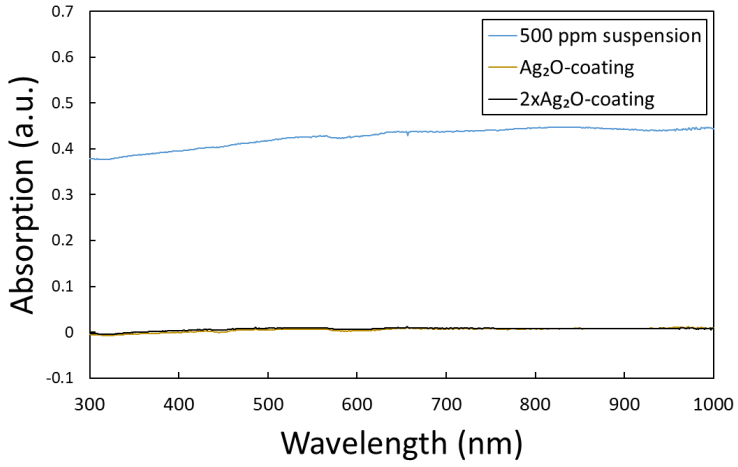

Figure S8 Test of attachment of particles. UV-Vis spectra of suspended particles after the coating was sonicated in ethanol. Any particles that are removed by sonication end up in suspension and reduce transmittance of light. Ag_2O suspension is shown for comparison. If all the particles were removed, there would be 170 ppm in the Ag_2O suspension and 340 ppm in the $2xAg_2O$ suspension. The absorption of a 500 ppm Ag_2O suspension is shown for comparison. The lack of absorption indicates that few particles were removed by sonication.

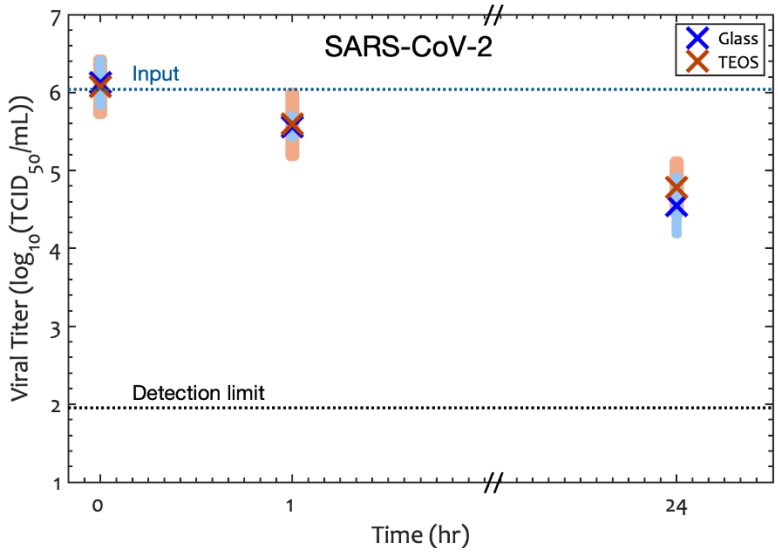

Figure S9 Comparison between SARS-COV-2 titer on TEOS-coated glass and uncoated glass over time. Shaded rectangles represent the 95% confidence interval and × represents the average of the log of the viral titer at each time point. There was not a significant difference for the TEOS-coated samples (Student's t-test, two tailed, unpaired, p= 0.82).

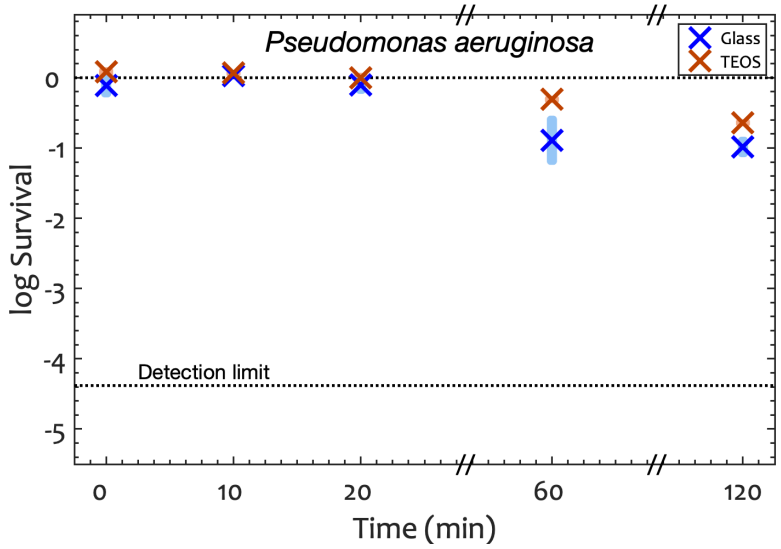

Figure S10 The Comparison of the survival of *P. aeruginosa* on TEOS and glass over time. Shaded rectangles represent the 95% confidence interval and × represents the average of the $\log_{10}$(Survival) at each time point. The results obtained from TEOS samples do not demonstrate a significant difference compared to glass (Student's t-test, two tailed, unpaired, p= 0.13).

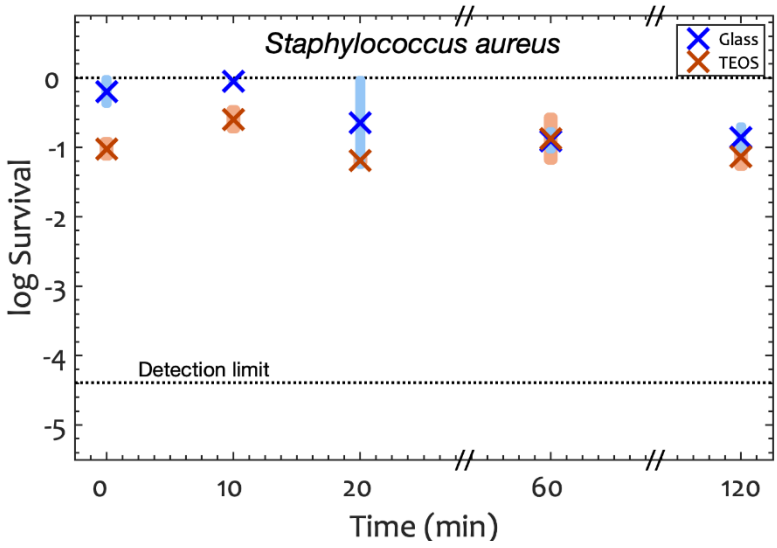

Figure S11 Comparison of the survival of *S. aureus* colony on TEOS and glass over time. Shaded rectangles represent the 95% confidence interval and × represents the average of the $\log_{10}$(Survival) at each time point. The results obtained from TEOS samples do not demonstrate a significant difference compared to glass (Student's t-test, two tailed, unpaired, p= 0.22).

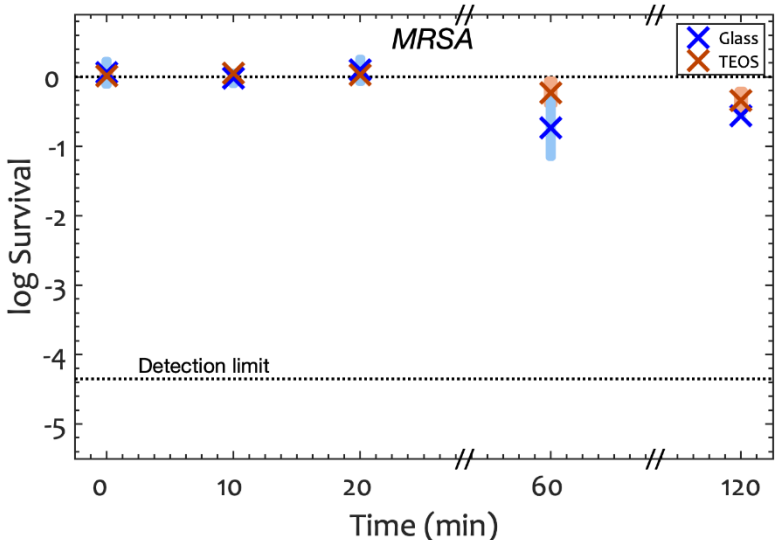

Figure S12 Comparison of the survival of MRSA on TEOS and glass over time. Shaded rectangles represent the 95% confidence interval and × represents the average of the $\log_{10}$(Survival) at each time point. The results obtained from TEOS samples do not demonstrate a significant difference compared to glass (Student's t-test, two tailed, unpaired, p= 0.64).

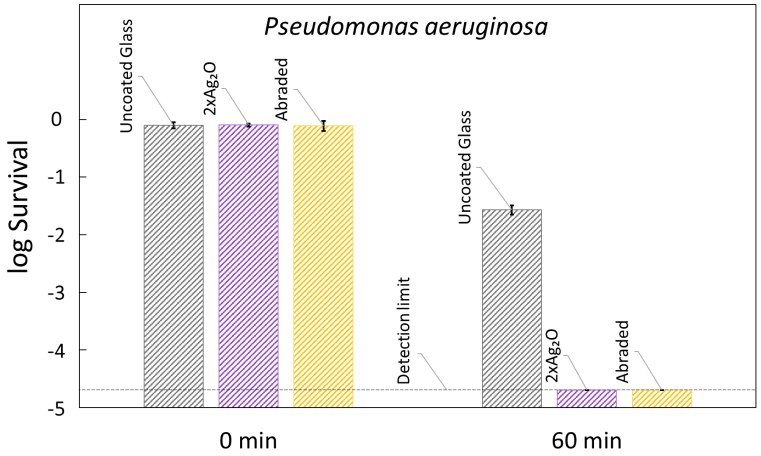

Figure S13 The effect of abrasion in the antibacterial properties of 2xAg_2O coating against *P. aeruginosa and* MRSA. The antibacterial properties remained unchanged after 10 abrasion cycles. Error bars show the 95% confidence intervals. The reduction after 1 h is greater than 99.9% on both the 2xAg2O and the abraded-2xAg_2O.

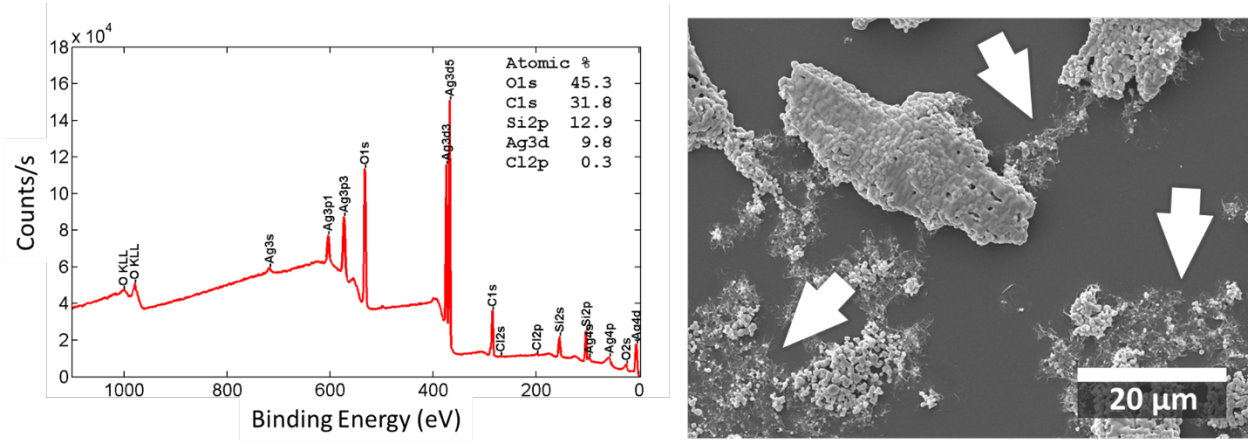

Figure S14 Characterization of M-2xAg_2O-coating. Left: elemental analysis results from XPS shows more silicon compared to 2xAg_2O-coating, which is in agreement with the procedure used and also the right side SEM image. Right: SEM image of the coating. Arrows show the presence of excess silica on the surface that leads to a higher level of adhesion of the particles on the coating.

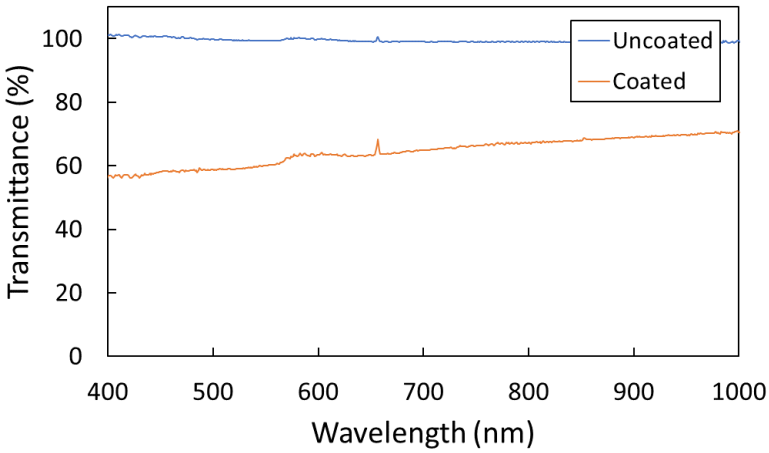

Figure S15 UV-Vis transmission spectrum of uncoated glass and M-2xAg_2O-coating shows that the films demonstrate more than 57-68% transparency in the visible range (400-700 nm). Results show that obtaining M-2xAg_2O-coating without a major loss in transparency of the 2xAg_2O-coating.

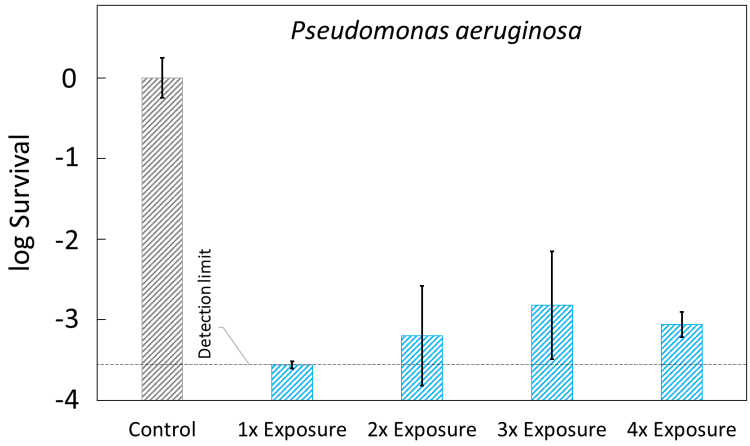

Figure S16 The effect of multiple bacteria exposure/sonicating/vortexing/titration/cleaning on M-2xAg_2O coating. 95% confidence intervals indicate more than 99.9% killing of *P. aeruginosa* was observed after 1 h with the bacteria in contact with the coating after each four cycles. Error bars show the 95% confidence intervals.

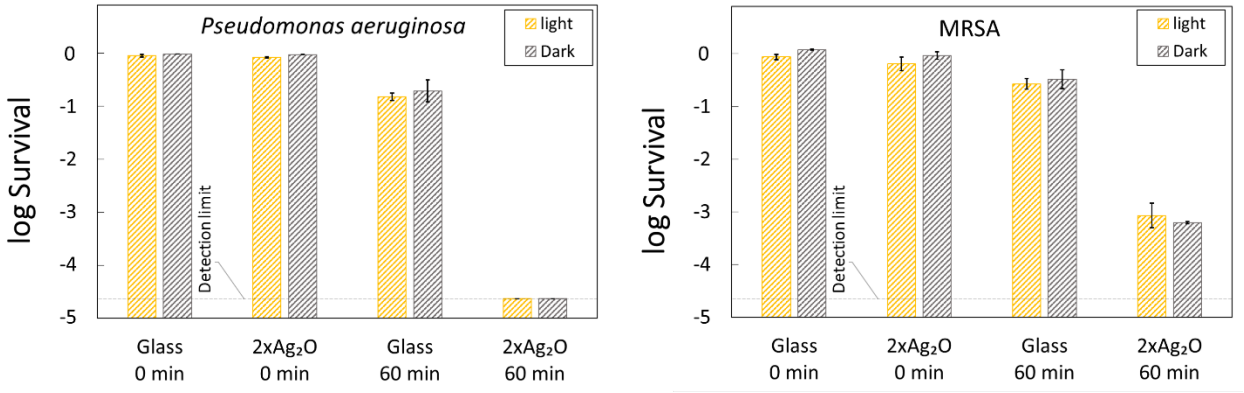

Figure S17 The effect of light on the antibacterial properties of the $2xAg_2O$ coating. *P. aeruginosa* and MRSA were tested. Student's t-test result did not show a significant difference between the CFU measurements in visible light and dark at 0 h or 1 hr (p>>0.05). Error bars show the 95% confidence intervals. Results show that the antibacterial activity of $2xAg_2O$ coating is retained in dark condition. Experiments in dark were done in darkness, except for the faint red light generated by the flame used to sterile microbiological tools. Experiments in the light were done with illumination by an Acuity LED light source within a biological safety cabinet.

Table S2 SARS-COV-2 $TCID_{50}$/mL assay results for Figure 4. The detection limit for the measurements is 90 $TCID_{50}$/mL and < indicates that the virus titer was less than the detection limit.

Material	Time	$TCID_{50}$/ml				log ($TCID_{50}$/ml)		
Nil (virus input control)		508079.8	508079.8	1495140		5.705	5.705	6.174
Glass	0	5080798	508079.8	1855376		6.705	5.705	6.268
		1606690	1166925	615552.8		6.205	6.067	5.789
	1 h	692703.4	194654.9	372663.3		5.840	5.289	5.571
		419371.2	472804.9	235829.8		5.622	5.674	5.372
	24 h	7824.056	34615.08	15587.41		3.893	4.539	4.192
		74575.92	50807.98	117846.5		4.872	4.705	5.071
TEOS	0 min	1606690	1656113	639634.6		6.205	6.219	5.805
	60 min	194654.9	508079.8	615552.8		5.289	5.705	5.789
	1 day	109462.5	41937.12	47280.49		5.039	4.622	4.674
Ag_2O-coating	0 min	1204847	615552.8	1326168		6.080	5.789	6.122
	60 min	61555.28	41937.12	50807.98		4.789	4.622	4.705
	1 day	1606.69	677.535	745.759		3.205	2.830	2.872
$2xAg_2O$-coating	0 min	132616.8	1326168	467969.6		5.122	6.122	5.670
	60 min	639.634	4366.591	6339.95		2.805	3.640	3.802
	1 day	<	<	<		<	<	<

Table S3 *Pseudomonas aeruginosa* CFU data for Figure 5. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		1205000	1145000	1255000		6.080	6.058	6.098
Glass	0	1285000	1315000	1425000		6.108	6.118	6.153
		1010000	1250000	1020000		5.777	5.869	5.781
	10	1325000	1285000	1245000		6.122	6.108	6.095
	20	1120000	1210000	1280000		5.822	5.855	5.880
		1225000	1255000	1245000		6.088	6.098	6.095
	60	105000	190000	175000		4.794	5.051	5.016
		160000	215000	-		5.474	5.602	-
	120	75000	60000	-		5.145	5.048	-
TEOS	0	1360000	1435000	1595000		6.133	6.156	6.202
	10	1330000	1430000	1460000		6.123	6.155	6.164
	20	1260000	1090000	1270000		6.100	6.037	6.103
	60	590000	635000	565000		5.770	5.802	5.752
	120	270000	260000	290000		5.431	5.414	5.462
Ag_2O-coating	0	1230000	1000000	1370000		6.089	6.000	6.136
		1970000	1895000	1905000		6.067	6.050	6.052
	10	635000	805000	735000		5.802	5.905	5.866
	20	243000	272000	189000		5.158	5.207	5.049
		245000	190000	190000		5.389	5.278	5.278
	60	<	<	<		<	<	<
		<	<	-		<	<	-
	120	<	<	-		<	<	-
2xAg_2O-coating	0	1135000	1425000	1325000		6.054	6.153	6.122
		1520000	1415000	1355000		5.954	5.923	5.904
	10	450000	435000	470000		5.653	5.638	5.672
	20	82500	106500	95000		4.689	4.800	4.750
		80000	70000	130000		4.903	4.845	5.113
	60	<	<	<		<	<	<
		<	<	-		<	<	-
	120	<	<	-		<	<	-

Table S4 *Staphylococcus aureus* CFU results for Figure 5. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		1075000	1265000	1355000		6.031	6.102	6.131
Glass	0	1245000	1260000	1375000		6.095	6.100	6.138
		130000	195000	135000		5.613	5.790	5.630
	10	1085000	1085000	1125000		6.03	6.035	6.051
	20	15000	20000	30000		4.676	4.801	4.977
		900000	1250000	1475000		5.954	6.096	6.168
	60	45000	35000	30000		5.153	5.044	4.977
		137650	132800	-		5.380	5.364	-
	120	83050	115850	-		5.160	5.305	-
TEOS	0	100000	145000	110000		5.000	5.161	5.041
	10	240000	330000	380000		5.380	5.518	5.579
	20	75000	75000	90000		4.875	4.875	4.954
	60	100000	210000	210000		5.000	5.322	5.322
	120	70000	110000	95000		4.845	5.041	4.977
Ag_2O-coating	0	305000	355000	410000		5.484	5.550	5.612
		575000	475000	660000		6.259	6.176	6.319
	10	225000	150000	235000		5.352	5.176	5.371
	20	191500	204500	146500		5.782	5.810	5.665
		80000	160000	105000		4.903	5.204	5.021
	60	2500	1000	500		3.897	3.500	3.198
		1800	1700	-		3.255	3.230	-
	120	2400	1300	-		3.380	3.113	-
2xAg_2O-coating	0	1410000	1515000	1565000		6.149	6.180	6.194
		210000	120000	150000		5.822	5.579	5.676
	10	340000	375000	645000		5.531	5.574	5.809
	20	186500	177000	66000		5.770	5.747	5.319
		35000	60000	55000		4.544	4.778	4.740
	60	250	250	250		2.897	2.897	2.897
		700	1200	-		2.845	3.079	-
	120	850	700	-		2.929	2.845	-

Table S5 MRSA CFU assay results for Figure 5. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		1130000	1005000	1265000		6.053	6.002	6.102
Glass	0	800000	860000	700000		5.903	5.934	5.845
		1265000	1400000	1395000		6.301	6.345	6.344
	10	1205000	1175000	900000		6.080	6.070	5.954
	20	1565000	1270000	1460000		6.394	6.303	6.364
		755000	760000	1195000		5.877	5.880	6.077
	60	120000	40000	55000		5.279	4.801	4.940
		530000	670000	-		5.724	5.826	-
	120	305000	310000	-		5.484	5.491	-
TEOS	0	1280000	1030000	1210000		6.107	6.012	6.082
	10	1345000	1275000	1205000		6.128	6.105	6.080
	20	1160000	1170000	1255000		6.064	6.068	6.098
	60	495000	705000	830000		5.694	5.848	5.919
	120	570000	390000	600000		5.755	5.591	5.778
Ag_2O-coating	0	995000	1345000	720000		5.997	6.128	5.857
		1565000	1435000	1835000		6.394	6.356	6.463
	10	755000	695000	545000		5.877	5.841	5.736
	20	497000	419000	431000		5.896	5.822	5.834
		650000	800000	350000		5.812	5.903	5.544
	60	3000	10500	5500		3.676	4.221	3.940
		6450	5400	-		3.809	3.732	-
	120	7850	8300	-		3.894	3.919	-
$2x Ag_2O$-coating	0	1370000	1215000	1935000		6.136	6.084	6.286
		1715000	1600000	1535000		6.434	6.403	6.385
	10	90000	115000	55000		4.954	5.060	4.740
	20	545000	630000	530000		5.936	5.999	5.924
		535000	375000	560000		5.728	5.574	5.748
	60	<	<	100		<	<	2.199
		1100	1150	-		3.041	3.060	-
	120	<	150	-		<	2.176	-

Table S6 *P. aeruginosa* CFU assay results for Figure 7. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		850000	835000	880000		5.929	5.922	5.944
Glass	0	605000	655000	665000		5.782	5.816	5.823
		620000	505000	610000		5.792	5.703	5.785
		445000	420000	700000		5.648	5.623	5.845
	20	585000	570000	450000		5.767	5.756	5.653
		325000	410000	535000		5.512	5.613	5.728
		545000	635000	675000		5.736	5.803	5.829
	60	33750	28550	33300		4.528	4.456	4.522
		45000	30000	45000		4.653	4.477	4.653
		13300	9000	11300		4.124	3.954	4.053
	120	11500	17000	16500		4.061	4.230	4.217
		6150	7650	5550		3.789	3.884	3.744
		8250	8950	5950		3.916	3.952	3.775
M-2xAg_2O-coating	0	655000	690000	660000		5.816	5.839	5.820
		695000	820000	920000		5.842	5.914	5.964
		790000	755000	720000		5.898	5.878	5.857
	20	142000	135000	131000		5.152	5.130	5.117
		90500	102000	97000		4.957	5.009	4.987
		4500	5000	2000		3.653	3.699	3.301
	60	<	<	<		<	<	<
		<	<	<		<	<	<
		<	<	<		<	<	<
	120	<	<	<		<	<	<
		<	<	<		<	<	<
		<	<	<		<	<	<

Table S7 MRSA CFU assay results for Figure 7. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		875000	920000	785000		5.942	5.964	5.895
		960000	1130000	1145000		5.982	6.053	6.059
		1865000	1300000	1595000		6.271	6.114	6.203
Glass	0	860000	765000	820000		5.934	5.884	5.914
		635000	385000	540000		5.803	5.585	5.732
		525000	1640000	935000		5.720	6.215	5.971
	20	675000	555000	555000		5.829	5.744	5.744
		580000	620000	770000		5.763	5.792	5.886
		1200000	1335000	975000		6.079	6.125	5.989
	60	274000	205500	262000		5.438	5.313	5.418
		133000	139000	109000		5.124	5.143	5.037
		304500	287500	307500		5.484	5.459	5.488
	120	143500	126000	136000		5.157	5.100	5.134
		46500	56000	39500		4.667	4.748	4.597
		154500	105500	176000		5.189	5.023	5.246
M-2xAg_2O-coating	0	715000	710000	590000		5.854	5.851	5.771
		727500	832500	780000		5.862	5.920	5.892
		1420000	1325000	1165000		6.152	6.122	6.066
	20	191500	23250	187000		5.282	4.366	5.272
		169000	126500	68500		5.228	5.102	4.836
		229500	209000	146500		5.361	5.320	5.166
	60	<	<	<		<	<	<
		<	<	<		<	<	<
		<	<	<		<	<	<
	120	<	<	<		<	<	<
		<	<	<		<	<	<
		<	<	<		<	<	<

Table S8 *P. aeruginosa* CFU assay results for Figure S13. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		2420000	2380000	1905000		6.384	6.377	6.280
		1060000	1280000	1040000		6.025	6.107	6.017
Glass	0	1895000	2015000	2415000		6.278	6.304	6.383
		1705000	1945000	2245000		6.232	6.289	6.351
		2030000	1825000	1810000		6.307	6.261	6.258
	60	85000	60000	60000		4.929	4.778	4.778
		70000	65000	85000		4.845	4.813	4.929
		60000	65000	60000		4.778	4.813	4.778
2xAg_2O-coating	0	2100000	1995000	1810000		6.322	6.300	6.258
		1820000	2300000	2125000		6.260	6.362	6.327
		1940000	1825000	2220000		6.288	6.261	6.346
	60	<	<	<		<	<	<
		<	<	<		<	<	<
		<	<	<		<	<	<
Abraded 2xAg_2O-coating	0	2250000	1935000	2280000		6.352	6.287	6.358
		1955000	1865000	1610000		6.291	6.271	6.207
		2170000	1810000	1635000		6.336	6.258	6.214
	60	<	<	<		<	<	<
		<	<	<		<	<	<
		<	<	<		<	<	<

Table S9 *P. aeruginosa* CFU assays results for Figure S17. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		1970000	2135000	2300000		6.294	6.329	6.362
Glass (light)	0	1665000	2260000	2065000		6.221	6.354	6.315
	0	1895000	1635000	1890000		6.278	6.214	6.276
	60	370000	360000	380000		5.568	5.556	5.580
	60	240000	255000	350000		5.380	5.407	5.544
2xAg_2O-coating (light)	0	1925000	1830000	1610000		6.284	6.262	6.207
	0	1860000	1525000	1865000		6.270	6.183	6.271
	60	<	<	<		<	<	<
	60	<	<	<		<	<	<
Glass (Dark)	0	1720000	2050000	2290000		6.236	6.312	6.360
	0	2320000	1825000	2010000		6.365	6.261	6.303
	60	740000	540000	635000		5.869	5.732	5.803
	60	200000	320000	300000		5.301	5.505	5.477
2xAg_2O-coating (Dark)	0	2290000	1905000	1865000		6.360	6.280	6.271
	0	1640000	2090000	2260000		6.215	6.320	6.354
	60	<	<	<		<	<	<
	60	<	<	<		<	<	<

Table S10 MRSA CFU assays results for Figure S17. The detection limit for the measurements is 50 CFU and < indicates that the bacterium colony forming units were less than the detection limit.

Material	Time (min)	CFU				log (CFU)		
Nil (Bacterium input control)		995000	1215000	1435000		5.998	6.085	6.157
Glass (light)	0	1240000	1035000	1180000		6.093	6.015	6.072
	0	835000	1175000	850000		5.922	6.070	5.929
	60	270000	215000	310000		5.431	5.332	5.491
	60	315000	450000	425000		5.498	5.653	5.628
2xAg_2O-coating (light)	0	865000	1060000	1110000		5.937	6.025	6.045
	0	715000	540000	540000		5.854	5.732	5.732
	60	700	750	500		2.845	2.875	2.699
	60	1500	2000	1500		3.176	3.301	3.176
Glass (Dark)	0	1215000	1660000	1570000		6.085	6.220	6.196
	0	1515000	1375000	1325000		6.180	6.138	6.122
	60	520000	745000	475000		5.716	5.872	5.677
	60	380000	275000	200000		5.580	5.439	5.301
2xAg_2O-coating (Dark)	0	725000	1120000	1110000		5.860	6.049	6.045
	0	1920000	1000000	1135000		6.283	6.000	6.055
	60	550	850	1050		2.740	2.929	3.021
	60	550	900	750		2.740	2.954	2.875

References

(1) Dey, B.; Engley, F. Methodology for Recovery of Chemically Treated Staphylococcus aureus with Neutralizing Medium. *Applied and Environmental Microbiology* **1983,** *45* (5), 1533-1537.