Enhancing Antibacterial Activity of Light-Activated Surfaces Containing Crystal Violet and ZnO nanoparticles: Investigation of Nanoparticle Size, Capping Ligand and Dopants

Supporting Information

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Supporting Information

1. Experimental

1.1 Material characterisation

TEM micrographs and energy dispersive X-ray spectra (EDX) were taken using a Jeol 2100 HR-TEM with a LaB_6 source operating at an acceleration voltage of 200 kV with an Oxford Instruments XMax EDS detector running AZTEC software. TEM samples were prepared by drop-casting the nanoparticle suspension (in hexane) onto a 400 Cu mesh lacy carbon film TEM grid (Agar Scientific Ltd). Micrographs were recorded onto a Gatan Orius charge-coupled device and analysed using ImageJ software.

Elemental Analysis was determined by Stephen Boyer at London Metropolitan University.

X-ray diffraction (XRD): was performed using an X'Pert Pro diffractometer (PANalytical B. V., The Netherlands) and X'Pert Data Collector software, version 2.2b. The instrument was used in the theta/theta reflection mode, fitted with a nickel filter, 0.04 rad Soller slit, 10 mm mask, 1/4° fixed divergence slit, and 1/2° fixed antiscatter slit. The diffraction patterns were analysed using Fityk (version 0.9.0; Marcin Wojdyr, 2010): the peaks were fitted to a SplitPearson7 function, and the particle size was calculated using the fitted full-width half-maximum using the Scherrer Equation.

For leaching experiments Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) analysis was conducted upon aqueous solutions (5 mL deionised water) in which a 1 cm^2 square of the modified polyurethane samples had been immersed for 2 hours or 2 days. The solutions were tested for [Zn] and [Mg], calibrating against stock solutions containing both Mg and Zn at 0.01, 0.1, 1 and 10 mg/mL and pure water. ICP-OES was measured on a Perkin Elmer Optima 2000 DV.

UV-vis absorption spectra of the modified polyurethane samples were taken using a Perkin Elmer Lambda 25 UV-vis spectrometer, within the range 300 – 700 nm (full range not shown). X-ray photoelectron spectroscopy (XPS) analysis of these samples were carried out using Thermo Scientific K-Alpha spectrometer using monochromated Al K*a* radiation. High resolution scans (0.1 eV) were collected at a pass energy of 50 eV, including the principal peaks of Zn (2p), Mg (1s), O (1s), N (1s) and C (1s). All binding energies were calibrated to the

C (1s) peak (284.5 eV). Water contact angle measurements obtained for all modified samples were prepared using a FTA 1000 Drop Shape Instrument. The average contact angle measurement over ≥10 measurements was calculated using a droplet of deionised water (~5 μL) dispensed by gravity from a gauge 30 needle, with a camera to photograph the samples side on. The data was analysed using FTA32 software. Polymers containing DOPA-capped nanoparticles were stored in a light box for an extended period of time to measure the photostability of CV when exposed to a white light emitting an average light intensity of 2800 ± 510 lux at a distance of 33 cm from the samples).
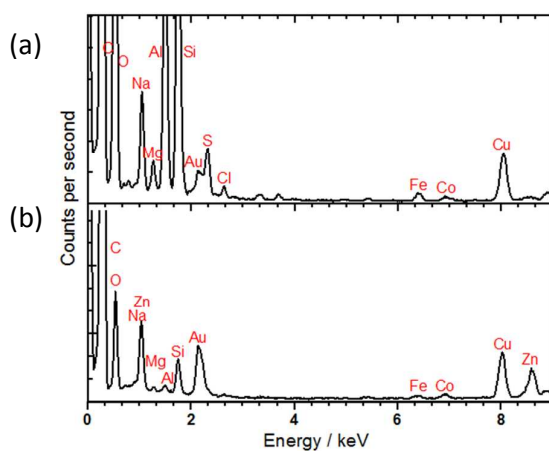
2. Results
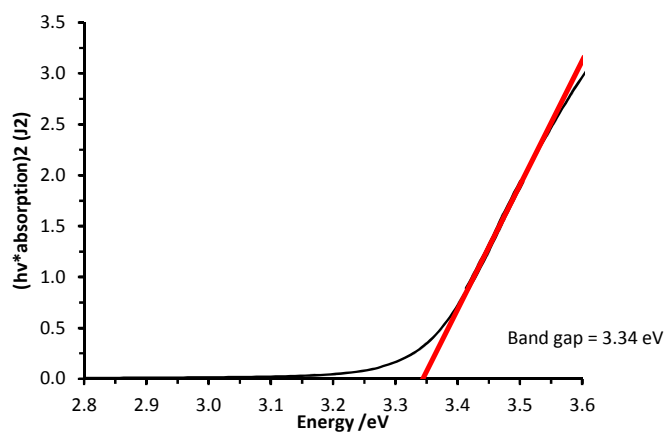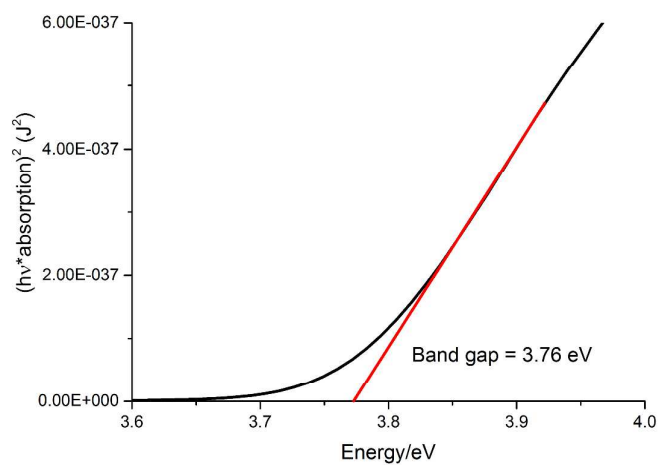
2.1 Energy dispersive X-ray

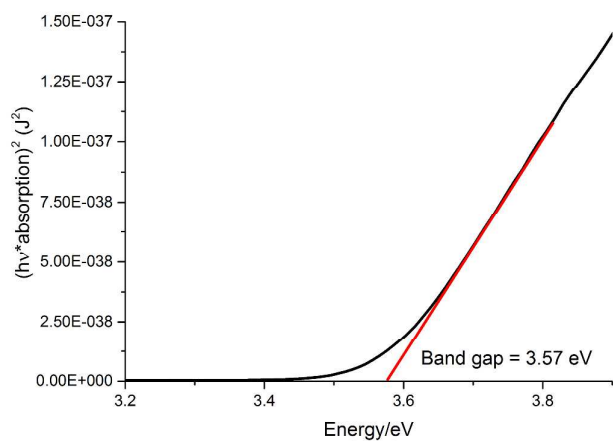**Figure S1** EDX spectrum of (a) MgO and (b) ZnMgO.

2.2 UV-vis absorbance spectroscopy analysis

Figure S2 Tauc plot to determine bandgap of ZnMgO capped with oleic acid (OA). The band onset was calculated as 3.34 eV.
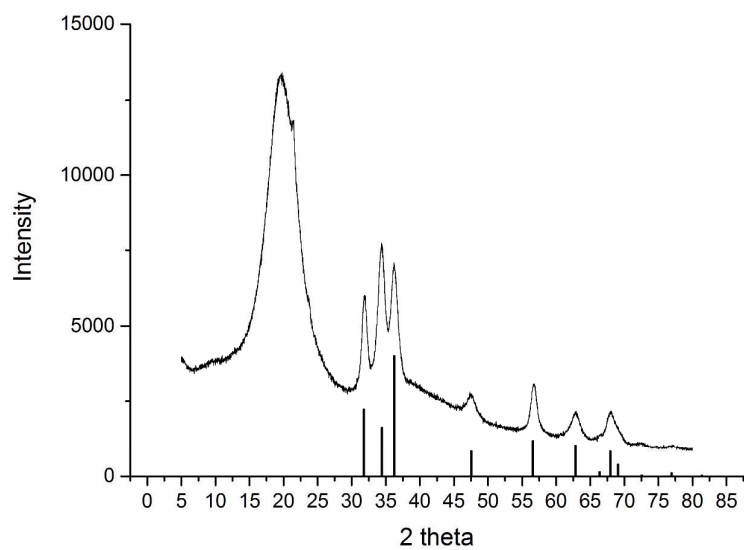

Figure S3 Tauc plot to determine bandgap of ZnO capped with dioctylphosphinate (DOPA). The band onset was calculated as 3.76 eV.
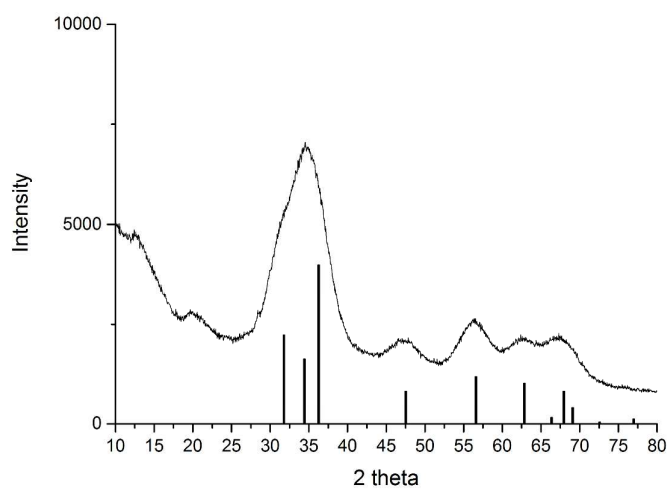
Figure S4 Tauc plot to determine bandgap of ZnMgO capped with dioctylphosphinate (DOPA). The band onset was calculated as 3.57 eV.
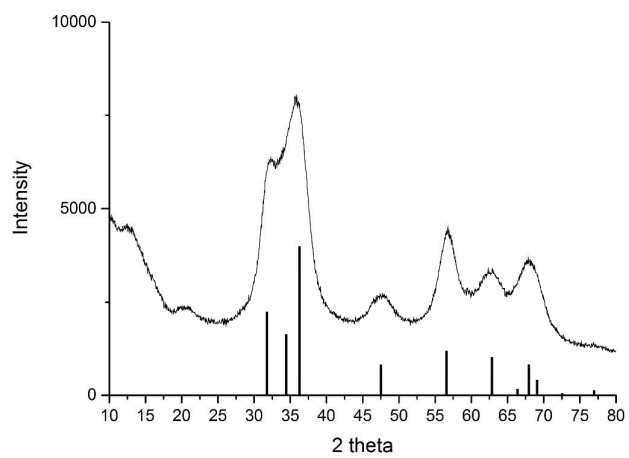
2.3 X-ray diffraction

Figure S5 XRD pattern for $Zn_{0.9}Mg_{0.1}O\_OA$. Large peak at 2 theta ~20° is from organic contaminants (likely to be excess oleic acid).
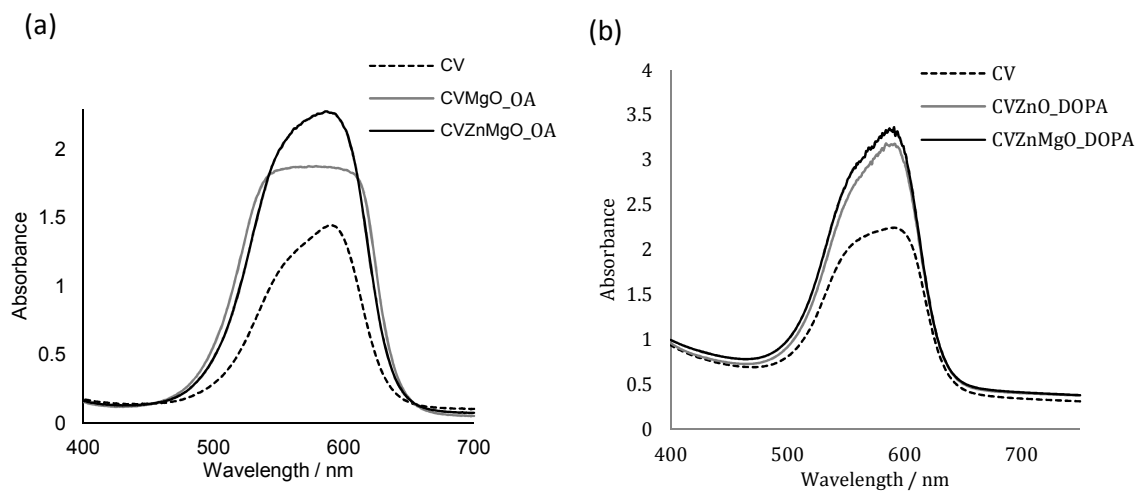
Figure S6 XRD pattern for ZnO_DOPA.

**Figure S7** XRD pattern for $Zn_{0.9}Mg_{0.1}O$_DOPA.

2.4 UV-vis Absorption Spectroscopy

(a)

(b)

Figure S8 UV-vis absorbance spectra measured in the range of 400 – 700 nm of samples used for microbiological investigation. (a) Samples incorporating oleic acid-capped ZnO: CV and ZnMgO encapsulated-polyurethane (CVZnMgO_OA) and CV and MgO encapsulated-polyurethane (CVMgO_OA). (b) Samples incorporating DOPA-capped ZnO: CV and ZnO-encapsulated polyurethane (CVZnO_DOPA) and CV and ZnMgO-encapsulated polyurethane (CVZnMgO_DOPA). CV indicates crystal violet-coated polyurethane.

2.5 X-ray Photoelectron Spectroscopy
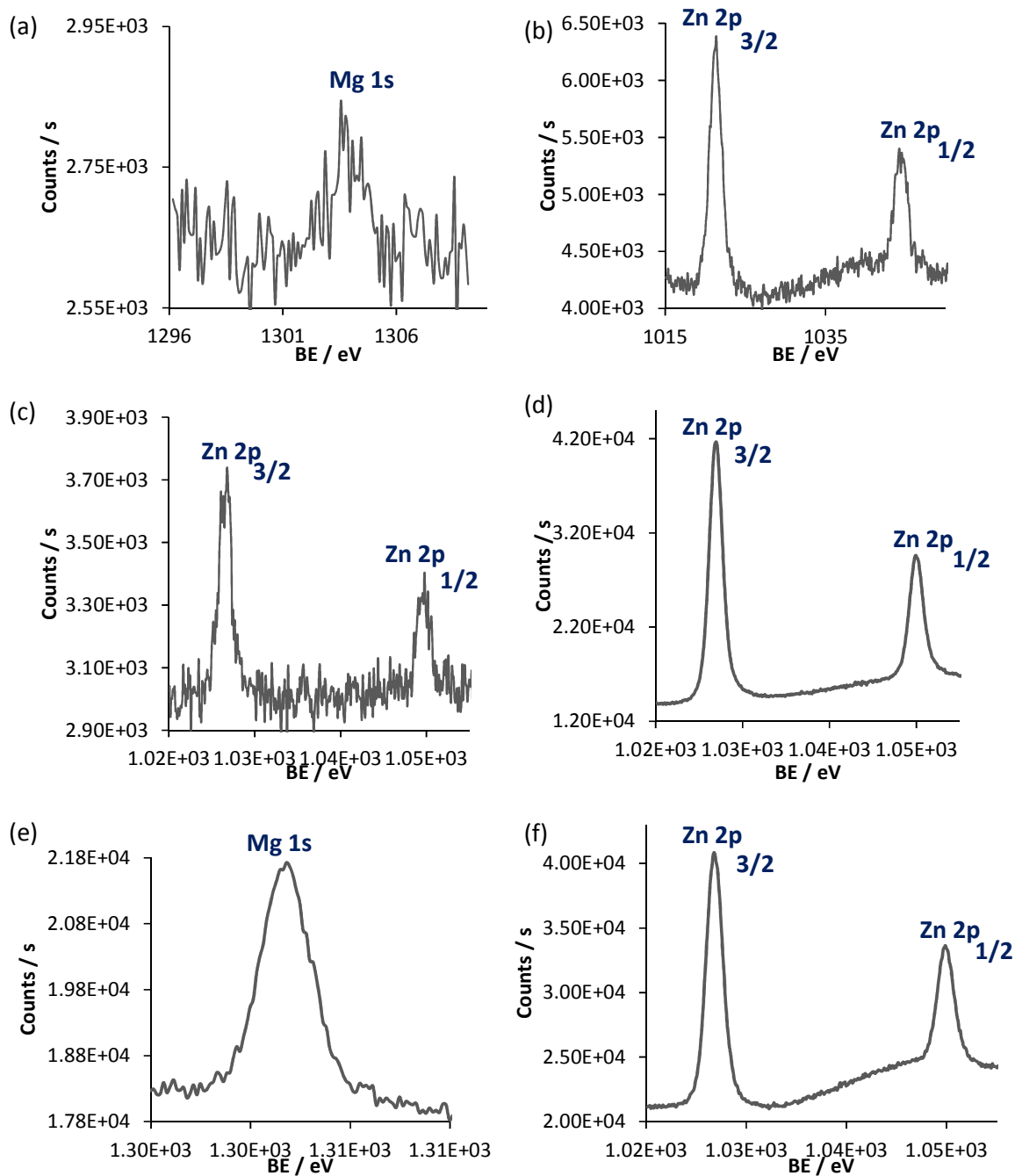
Figure S9 XPS spectra for modified polymer samples: (a) Mg (1s) on CVMgO_OA surface, (b) Zn (2p) on CVZnMgO_OA surface, (c) Zn (2p) on CVZnMgO_OA sputtered 50 s, (d) Zn (2p) on CVZnMgO_DOPA 50 s, (e) Mg 1(s) on CVZnMgO_DOPA sputtered 50 s and (f) Zn (2p) on CVZnO_DOPA sputtered 50 s.

2.6 Water Contact Angle measurements

Polymer sample	Contact angle (^{o})	Standard deviation
Untreated	93	± 1.1
Control (DCM/hexane)	93	± 1.2
MgO_OA	93	± 1.4
ZnMgO_OA	97	± 0.5
ZnMgO_DOPA	95	± 0.7
ZnO_DOPA	99	± 0.4
CV	101	± 1.3
Control (toluene)	94	± 0.8
CVMgO_OA	93	± 0.5
CVZnMgO_OA	97	± 1.4
CVZnMgO_DOPA	98	± 0.7
CVZnO_DOPA	95	± 1.0

Table S1 Water contact angle measurements of samples used in the microbiological testing: untreated, control (treated with DCM and hexane, metal oxide nanoparticle-encapsulated (MgO_OA / ZnMgO_OA / ZnO_DOPA / ZnMgO_DOPA), control (treated with toluene), crystal violet-coated (CV), and crystal violet-coated and metal oxide nanoparticle-encapsulated (CVMgO_OA / CVZnMgO_OA / CVZnO_DOPA / CVZnMgO_DOPA).

## 2.7 Photostability of Polymer containing DOPA-capped Nanoparticles
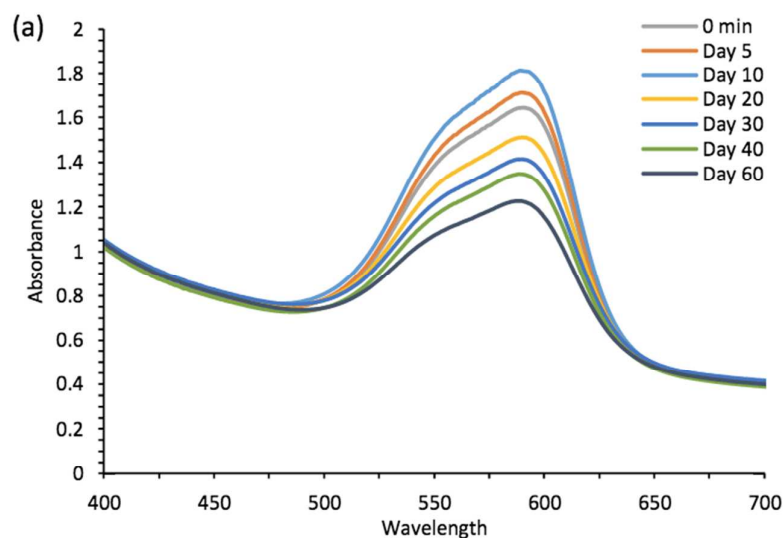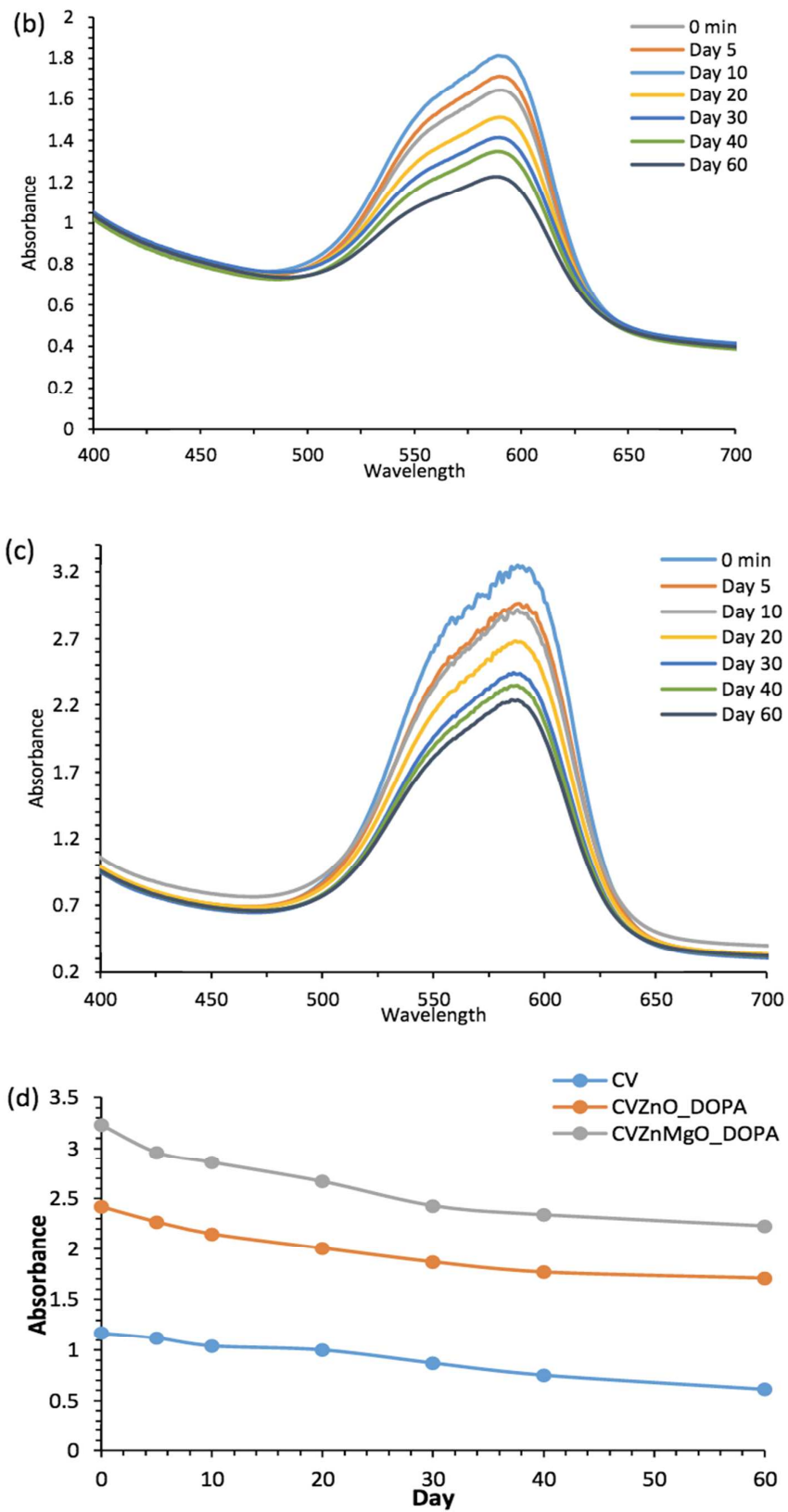
Figure S10 UV-vis absorbance spectra of (a) crystal violet-coated (CV), (b) crystal violet and DOPA-capped ZnO-incorporated (CVZnO_DOPA) and (c) crystal violet and DOPA-capped ZnMgO-incorporated (CVZnMgO_DOPA)

polymers. The samples were exposed to a white light source emitting an average light intensity of 3880 ± 200 lux at a distance of 33 cm from the samples. (d) The rate of photodegradation of the samples upon (60 days; 3880 lux) was displayed as a change in absorbance at the CV absorbance maxima over time. Data shown with control polymer readings subtracted.

2.8 Microbiological Investigation
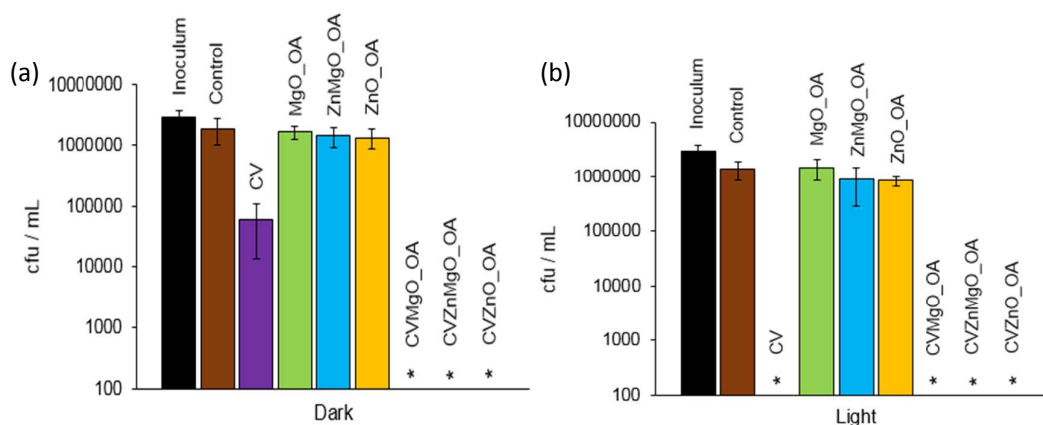
Figure S11 Viable counts of *S. aureus* after incubation at 20°C on modified polyurethane squares for: (a) 2 h in the dark and (b) 2 h exposure to white light illumination with an average light intensity of 6600 ± 900 lux at a distance of 25 cm from the samples. * indicates bacterial counts were reduced to below the detection limit of 100 cfu/mL. OA indicates nanoparticles synthesised with an oleic acid capping. Error bars are based on the standard deviation of three experimental replicates.
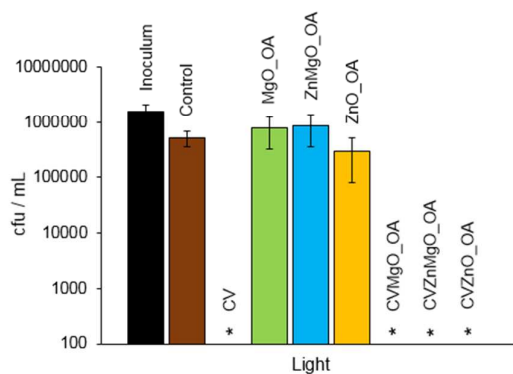
Figure S12 Viable counts of *E. coli* after incubation at 20°C on modified polyurethane squares for 4 h exposure to white light illumination with an average light intensity of 6600 ± 900 lux at a distance of 25 cm from the samples. * indicates bacterial counts were reduced to below the detection limit of 100 cfu/mL. OA indicates nanoparticles synthesised with an oleic acid capping. Error bars are based on the standard deviation of three experimental replicates.

Leaching study

1 cm² polyurethane squares impregnated with NPs were placed in 2 mL of de-ionised water for periods of 2 hours or 2 days. After removal of the polymer sample the aqueous solutions were diluted to 5 mL and analysed by ICP-OES for [Zn] and [Mg] against calibration curves for Mg and Zn.

ICP results	SD = standard deviation, all data in mg/L							
	2 hours				**2 days**			
	[Zn]	SD	**[Mg]**	SD	**[Zn]**	SD	**[Mg]**	SD
ZnO_DOPA	**0.083**	0.001	**0.003**	0.0003	**0.169**	0.005	**0.004**	0.003
ZnMgO_DOPA	**0.081**	0.001	**0.009**	0	**0.268**	0.0004	**0.008**	0.0002
ZnO_OA	**0.132**	0.0002	**0.002**	0.0003	**1.696**	0.017	**0.006**	0.0003
ZnMgO_OA	**0.095**	0.001	**0.009**	0.001	**0.337**	0.004	**0.01**	0.0001
MgO_OA	**0.012**	0.002	**0.027**	0.001	**0.026**	0.003	**0.03**	0.0002

Table S2 Amount of leaching (mg/L) of Zn^{2+} and/or Mg^{2+} ions from modified polymer samples after 2 hours and 2 days.

Calculation of DOPA surface coverage of small ZnO/ZnMgO nanoparticles

Both ZnO@DOPA and ZnMgO@DOPA nanoparticle samples were analysed by elemental analysis (EA) and thermogravimetric analysis (TGA) to determine the ligand content.

ZnO@DOPA. C% by elemental analysis = 25.3% , loss of alkyl chains by TGA = 29 wt%

Estimated metal:ligand ratio from EA = 5.8:1, from TGA = 5.9:1

ZnMgO@DOPA. C% by elemental analysis = 25.4%, loss of alkyl chains by TGA = 32 wt %

Estimated metal:ligand ratio from EA ~ 6:1, from TGA = 5.4:1

From the sizes established by XRD analysis and these ratios an estimate of the ligand coverage can be made.

To calculate the amount of ligand required to fully coat a nanoparticle surface certain assumptions were used:

1. The nanoparticles are perfectly spherical

2. The density (ρ) of the particles matches that of the bulk phase material (e.g. ρ(ZnO) = 5.61 g/cm³ and density $\rho(Zn_{0.9}Mg_{0.1}O)$ ~ 5.33 by replacement of 10% Zn by Mg in the same structure)

3. The ZnO particles are pure ZnO (with no accounting for substoichiometric oxygen or dangling bonds)

4. The ligands pack perfectly across the surface with a contact area (C.A.) of 0.244 nm^2 for DOPA.[1]

Using these assumptions the surface area and volume of a nanoparticle was calculated:

S.A. = $4\pi r^2$ (*r = radius of nanoparticle*)

Vol = $^4/_3\pi r^3$

From the volume and density the no. of metal atoms (moles) was determined:

n_{metal} = vol x ρ/m (*where m = atomic mass of subunit eg. Cu or ZnO*)

From the surface area the number of ligands (moles) required for full coverage was determined:

n_{ligand} = S.A. / C.A.$_{ligand}$

The ratio of these two values gives the required metal to ligand ratio for full coverage.

e.g. for a ZnO NP of r = 1.1 nm, n_{metal} = 3.75×10^{-22} (225 ZnO units) and n_{ligand} = 1.03×10^{-22} (62 ligands) for DOPA. Therefore a metal:ligand ratio of 3.6:1 is required for complete coverage of a d = 2.2 nm ZnO particle.

The empirically calculated 6:1 ratio corresponds to a 60% coverage of the ZnO surface.

e.g. for a $Zn_{0.9}Mg_{0.1}O$ NP of r = 1.4 nm, n_{metal} = 7.93×10^{-22} (477 MO units) and n_{ligand} = 1.68×10^{-22} (101 ligands) for DOPA. Therefore a metal:ligand ratio of 4.7:1 is required for complete coverage of a d = 2.8 nm ZnMgO particle.

The empirically calculated 6:1 ratio corresponds to a 78% coverage of the ZnO surface.

References

1. Cooper, R.J.; Camp, P.J.; Henderson, D.K.; Lovatt, P.A.; Nation, D.A.; Richards, S.; Tasker, P.A. The binding of phosphonic acids at aluminium oxide surfaces and correlation with passivation of aluminium flake. *Dalton Trans.,* **2007**, 10, 1300.