

Supporting information

(15 pages, 15 figures, 2 tables)

Antibacterial Surfaces with Activity Against Antimicrobial Resistant Bacterial Pathogens and Endospores

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Supplementary information

1. Experimental methods

1.1 Material preparation and characterisation

1.1.1 ZnO nanoparticle preparation

All chemicals were used directly from suppliers. The Schlenk line and glovebox techniques were used to manipulate air sensitive chemicals (diethyl zinc is pyrophoric, used with caution). Liquid chemicals (e.g. diethyl zinc, oleic acid, linoleic acid), were measured by negative weight of donor flask. Toluene was pre-dried over potassium hydroxide and then further dried by refluxing over sodium, then degassed by freeze pump thaw techniques and stored under nitrogen.

ZnO nanoparticles were prepared with oleate, linoleate and stearate ligands following an established route.¹ A carboxylic acid (oleic acid, linoleic acid or stearic acid) was placed into a Schlenk flask with a stirrer and dried under vacuum. Dry toluene was added to the ligand in a glovebox (for the more soluble oleic and linoleic acids, half the quantity of solvent used for a typical stearic acid synthesis was used) and then either 5 or 10 equivalents of diethyl zinc (ZnEt_2) was added dropwise to the solution whilst stirring. A 0.4 M solution of water in acetone was added to the mixture to hydrolyse the organometallic precursors to ZnO. The final nanoparticle suspensions were precipitated by adding acetone, centrifuged and washed by toluene/acetone and acetone with subsequent centrifugation steps. The particles were air dried overnight. To halt any ripening which may occur in the solid state the particles should be stored under N_2 gas or vacuum.²

Sample	ZnEt_2 (mg, mmol)	Carboxylic acid (mg, mmol)	Toluene (mL)	Water (μL , mmol)	Acetone (mL)	Yield (mg, %)
ZnO_oleate(5:1)	655, 5.30	300, 1.06	17.7	191, 10.6	26.6	650, 89
ZnO_oleate(10:1)	400, 3.24	91.5, 0.32	10.4	117, 6.5	16.2	306, 86
ZnO_linoleate(5:1)	400, 3.24	181.7, 0.65	10.4	117, 6.5	16.2	297, 67
ZnO_stearate(5:1)	500, 4.05	230, 0.81	27	146, 8.1	20.2	505, 90

1.1.2 ZnO nanoparticle characterisation

ZnO nanoparticles were characterised by IR spectroscopy (Fig S1-4), powder X-ray diffraction (Fig S5), UV spectroscopy (Fig S6 and S7) and elemental microanalysis (Table S1).

Solid-state Fourier Transform Infra-red (FT-IR) spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer with a Universal ATR Sampling Accessory. 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. U.V. spectroscopy was recorded using a PerkinElmer Lambda 950 spectrophotometer, from toluene solutions. Elemental micro-analysis (EA) was determined by Stephen Boyer at London Metropolitan University. Thermogravimetric analysis was undertaken under an air atmosphere, using a Mettler/Toledo TGA/DSC 1LF/UMX instrument at a heating rate of 10K/min.

1.1.3 Polymer preparation

1 cm² polymer squares were immersed into swelling solutions containing toluene for incorporating ZnO nanoparticles (1 mg/mL). The polymer samples were left to swell-encapsulate for 24 hr and then dipped into 0.001 M aqueous crystal violet solution in water for 72 hr (dark conditions, RT). For the antibacterial investigation, control samples were prepared (solvent treated only) and polymer containing only ZnO nanoparticles or crystal violet alone.

1.1.4 Polymer characterisation

UV-vis absorption spectra of the modified polymer samples were measured in the range 300 – 750 nm using a PerkinElmer Lambda 25 UV-vis spectrometer (full range not shown). A Thermo K-Alpha spectrometer using monochromated Al K α radiation was used to carry out X-ray photoelectron spectroscopy (XPS) of modified polymer samples. High-resolution scans (0.1 eV) were collected at a pass energy of 50 eV, including principal peaks of Zn (2p), Mg (1s), O (1s), N (1s) and C (1s), at the emission angle of 90° to the surface. A depth profile was measured at the surface of the polymer and sputtered for 50 seconds, which corresponds to approximately 10 nm depth. The sputtering was done perpendicular to the surface and the zinc was measured from the survey scan. All binding energies were calibrated to the C (1s) peak at 284.5 eV. All CV-coated polymers were stored in a light box for an extended period of time to measure the photo-degradation of the dye when exposed to a white light source emitting an average light intensity of 2800 \pm 510 lux (distance of 33 cm from the samples).

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 cm² 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.

1.1.5 Ligand variation investigation

ZnO nanoparticles were synthesised with different ligands to see if oleate capping was required for bactericidal activity. In addition, oleate-capped ZnO (5:1) was synthesised again but with a greater exposure of ZnO surface (10:1). The following nanoparticles were synthesised using similar methods as described in section 1.2, supporting information: 3.6 nm stearate-capped ZnO (5:1), 4.0 nm linoleate-capped ZnO (5:1), and 4.9 nm oleate-capped ZnO (10:1).

2. Results

2.1. FTIR Spectroscopy of ZnO nanoparticles

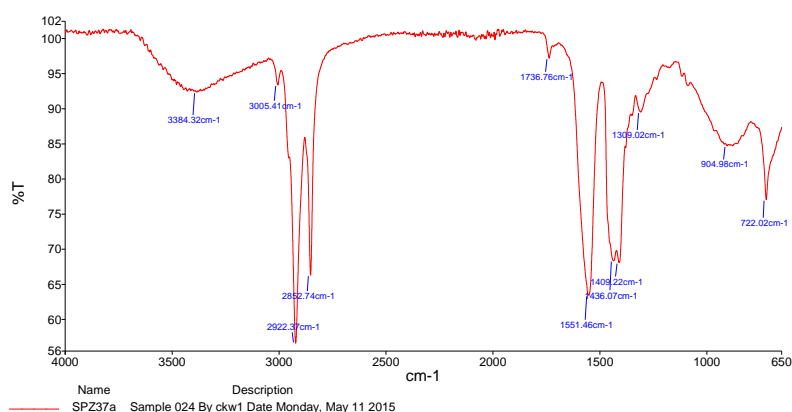


Fig. S1 FTIR spectrum of ZnO_{oleate} (5:1).

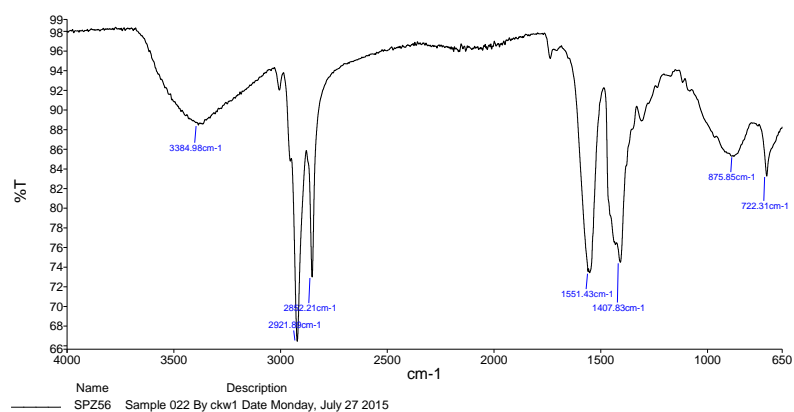


Fig. S2 FTIR spectrum of ZnO_oleate (10:1).

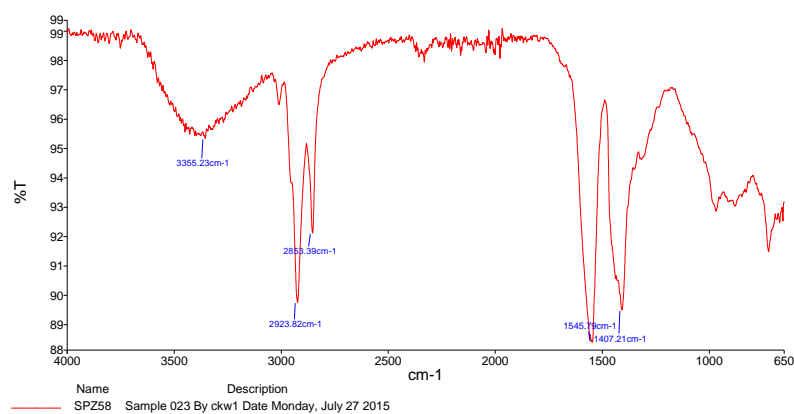


Fig. S3 FTIR spectrum of ZnO_linoleate (5:1).

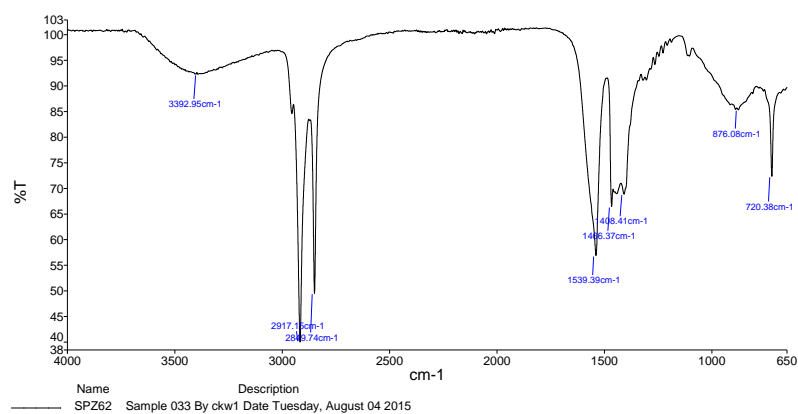


Fig. S4 FTIR spectrum of ZnO_stearate (5:1).

2.2. XRD patterns of ZnO nanoparticles

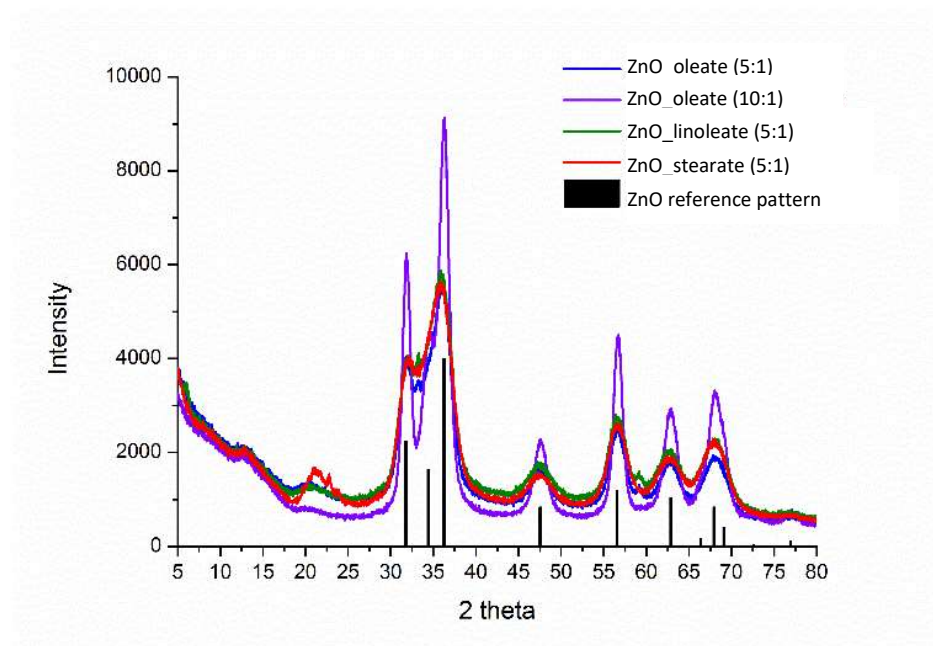


Fig. S5 Powder XRD patterns of ZnO nanoparticles with oleate, linoleate and stearate ligands. The size of the particles may be estimated by analysis of the peak width using the Scherrer equation. The signals at $2\theta = 47.5$ and 56.6 were analysed for each batch of nanoparticles giving sizes of: ZnO_oleate (5:1), 2.8-4.1 nm (over three batches); ZnO_oleate (10:1), 4.9-7.4 nm; ZnO_linoleate (5:1), 2.8-3.6 nm, ZnO_stearate (5:1), 2.8-3.3 nm. The broad signals at $2\theta = 18-25^\circ$ are expected to be from the organic ligands, in the case of ZnO_stearate a minor trace of impurity $\text{Zn}(\text{stearate})_2$ may also be present (indicated by sharper lines).¹

2.3. UV spectroscopy of ZnO nanoparticles

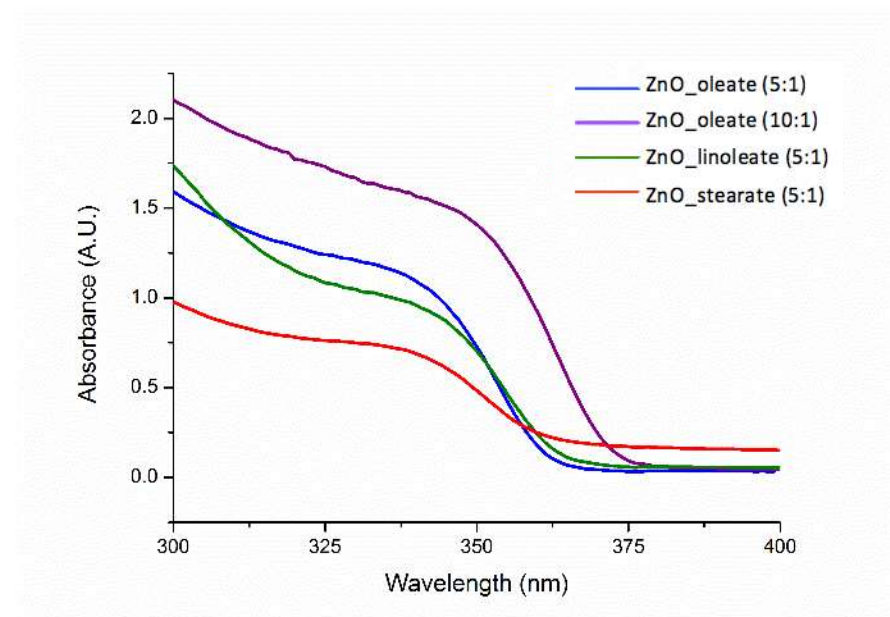


Fig. S6 UV spectra of ZnO nanoparticles dissolved in toluene (approx 0.4 mg/mL). An estimate of the particle size can be made by the onset of UV absorption.³ Sizes were calculated from the maximum gradient of the UV absorption onset corresponding to: ZnO_oleate (5:1), 3.7-3.9 nm (over three batches) nm; ZnO_oleate (10:1), 4.9 nm; ZnO_linoleate (5:1), 4.0 nm, ZnO_stearate (5:1), 3.6 nm. All sizes are a close match for the sizes estimated by XRD (see Fig S5).

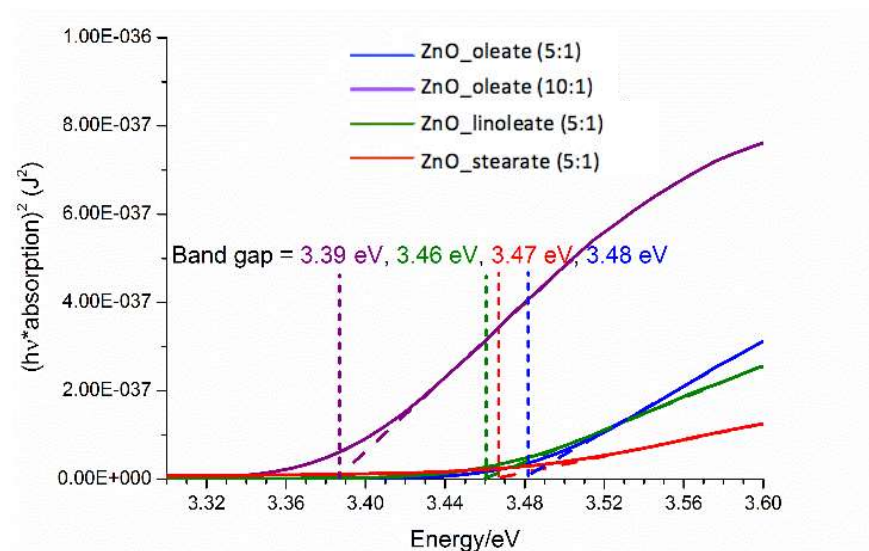


Fig. S7 Tauc plots derived from UV absorption spectra of ZnO nanoparticles (see Fig S5). Band gaps determined: ZnO_oleate (5:1), 3.48 ± 0.01 eV nm; ZnO_oleate (10:1), 3.39 ± 0.01 eV; ZnO_linoleate (5:1), 3.46 ± 0.01 eV, ZnO_stearate (5:1), 3.47 ± 0.01 eV.

2.4. Elemental microanalysis of ZnO nanoparticles, ligand weight percentage and surface coverage

ZnO nanoparticles were dried under vacuum ($\sim 10^{-2}$ bar) overnight and submitted for elemental microanalysis. The carbon percentage was calculated for each sample, from this the weight % of ligand could be established. The calculated metal:ligand ratio deviates slightly from the original stoichiometry due to the loss of some ligand (likely in the form of $\text{Zn}_4\text{O}(\text{carboxylate})_6$ clusters during the workup

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

- The nanoparticles are perfectly spherical
- The density (ρ) of the particles matches that of the bulk phase material (e.g. $\rho(\text{ZnO}) = 5.61 \text{ g/cm}^3$)
- The ZnO particles are pure ZnO (with no accounting for substoichiometric oxygen or dangling bonds)
- The ligands pack perfectly across the surface with a contact area (C.A.) of 0.2 nm^2 for all carboxylates.^{1,4,5}

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

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

$$\text{Volume} = \frac{4}{3}\pi r^3$$

From the volume and density, the number of metal atoms (moles) was determined:

$$n_{\text{metal}} = \text{vol} \times \rho / m \text{ (where } m = \text{atomic mass of subunit eg. ZnO)}$$

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

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

The ratio of these two values gives the required metal : ligand ratio for full coverage. Comparing this ratio to the calculated metal:ligand ratio allows an estimation of the amount of surface coverage of the nanoparticles using the particle sizes estimated by UV spectroscopy.

The surface area per mg ZnO_ligand was established by: $(\text{Weight \% ZnO}/100) \times \text{S.A.} / (\text{Volume} \times 5.61 \text{ g/cm}^3)$.

$$\text{e.g. for ZnO}_{\text{oleate}}(10:1) \text{ S.A./g} = 0.745 \times 75.4 \text{ nm}^2 / (61.6 \text{ nm}^3 \times 5.61) = 1.6 \times 10^{-17} \text{ nm}^2 \text{ (or } 1.6 \text{ m}^2)$$

Table S1. Analysis of the composition of the produced ZnO nanoparticles. Surface coverage determined for a typical size nanoparticle as expected from analysis of UV spectra.

Sample	Weight % of ligand	Metal:ligand ratio	Surface Coverage
ZnO_oleate(5:1)	39.0-39.7 (over three batches)	5.3-5.4:1	95-99% (Size 3.8-3.9 nm)
ZnO_oleate(10:1)	25.5	10.1:1	66% (Size 4.9 nm)

ZnO_linoleate(5:1)	35.3	6.3:1	86% (Size 4.0 nm)
ZnO_stearate(5:1)	40.3	5.2:1	93% (Size 3.6 nm)

2.5 UV-Vis Absorbance Spectroscopy of polymer samples

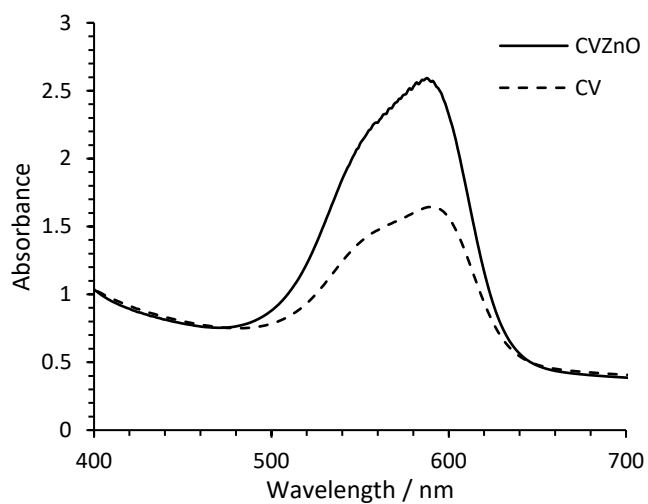


Fig. S8 UV-vis absorption spectra measured in the range of 400 – 700 nm of modified polyurethane samples: crystal violet-coated polyurethane (CV) and crystal violet and ZnO-encapsulated polyurethane (CVZnO).

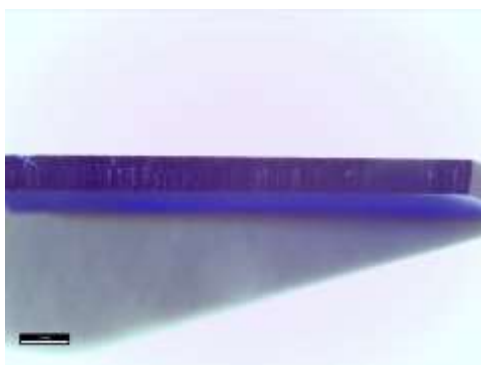


Fig. S9. Microscope image of crystal violet-coated zinc oxide encapsulated polyurethane sample cross section, to show crystal violet penetration through the polymer bulk. Scale bar is 1 mm

2.6 Photostability of CV and CVZnO samples

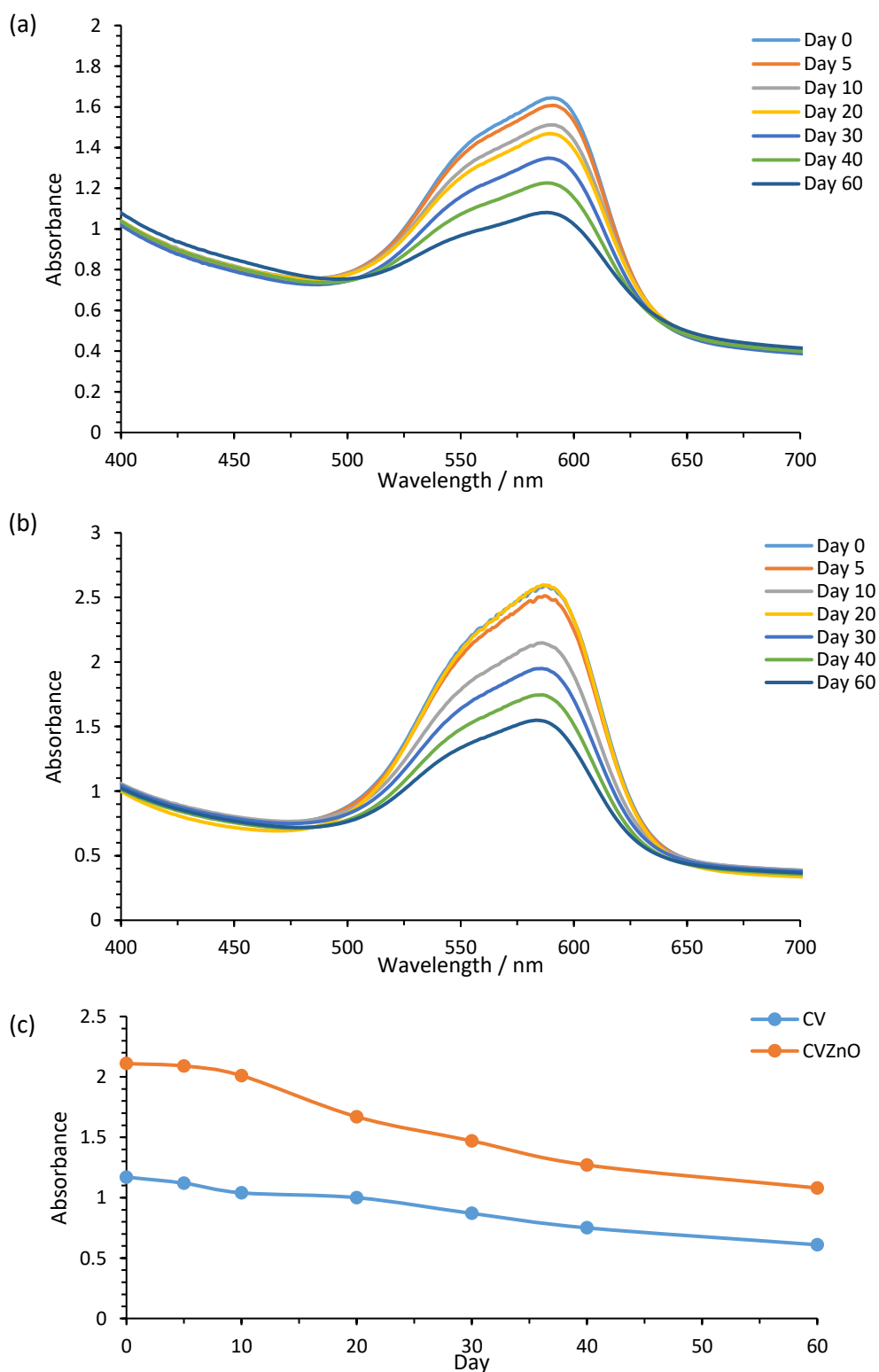
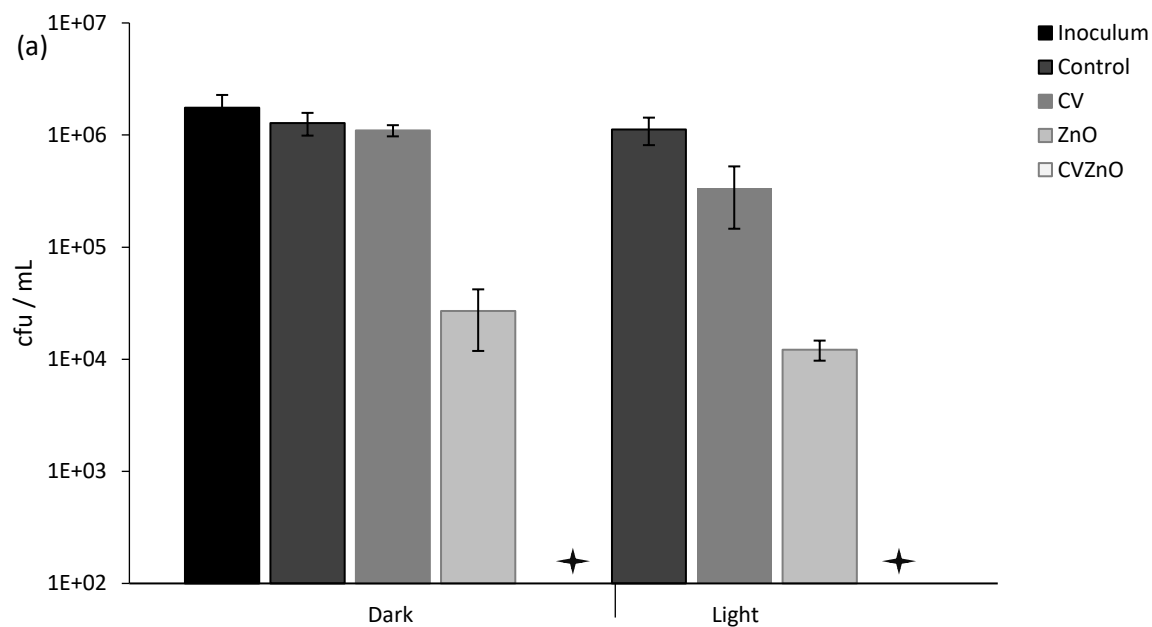


Fig. S10 UV-vis absorbance spectra measured in the range 400 – 700 nm of (a) crystal violet-coated polymer and (b) crystal violet and ZnO-incorporated polymer. The samples were exposed to a white light source emitting an average light intensity of 3880 ± 200 lux at a distance of 35 cm from the samples. (c) The rate of photodegradation of CV- and CVZnO-coated polymers upon exposure to a high lux intensity white light source (60 days; 3880 lux) displayed as a change in absorbance at the crystal violet absorbance maxima over time. Data shown with control polymer readings subtracted.

2.7 Induced coupled plasma-optical emission spectroscopy (ICP-OES)

ICP-OES was measured on a Perkin Elmer Optima 2000 DV. 1 cm² ZnO-incorporated polyurethane samples were immersed in 2 mL deionised water for up to 2 – 48 hours. This solution was diluted to 5 mL and the zinc concentration was calculated using a calibration curve determined from solutions containing [Zn] = 0, 0.1, 1 and 10 mg/L.

2.8 Antibacterial Activity against *E. coli* ATCC 25922 and MRSA 4742



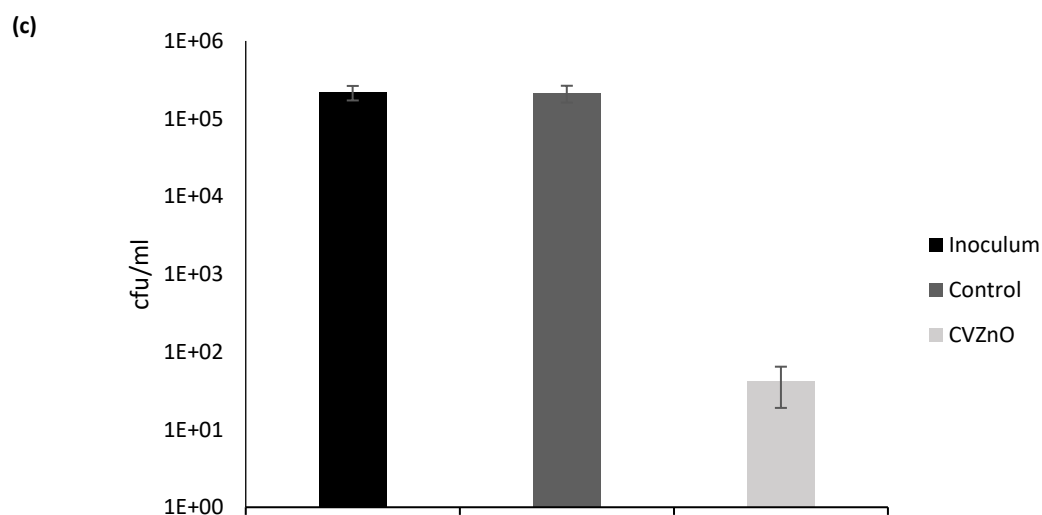
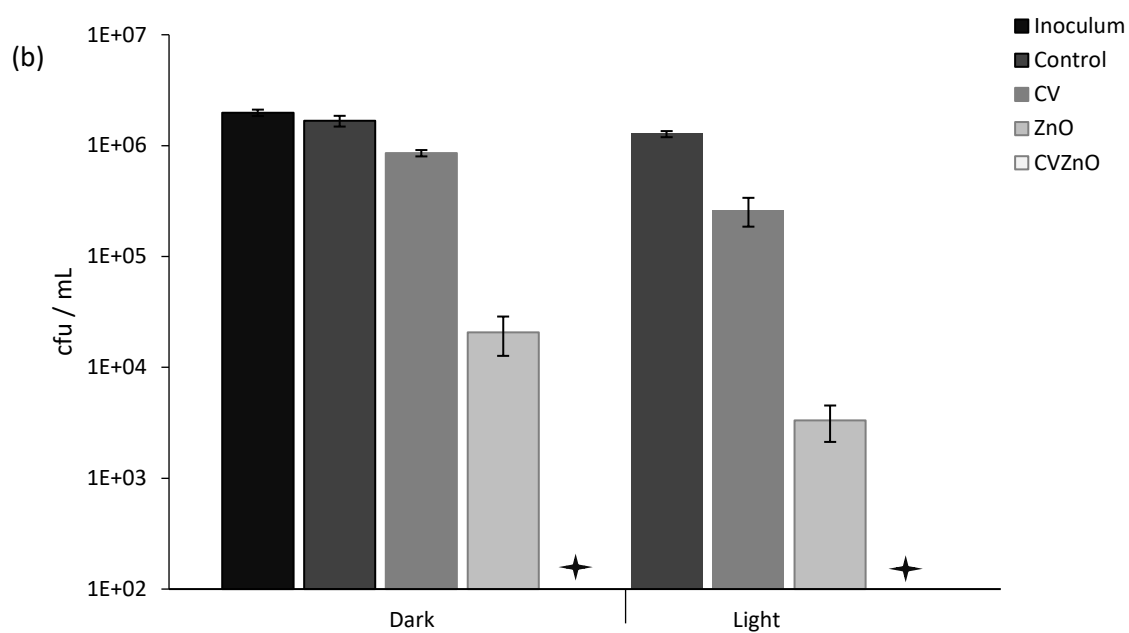


Fig. S11 Viable counts of *E. coli* ATCC 25922 after incubation at 20°C on modified polyurethane squares for: (a) 2 h and (b) 3 h exposure to white light (500 ± 300 lux) and in the dark. ◆ indicates bacterial counts were reduced to below the detection limit of 100 cfu/mL. (c) Viable counts of MRSA 4742 after incubation at 20°C on modified polyurethane squares for 2 h exposure to white light (500 ± 300 lux).

2.9 Variation in ligands: stearic acid, oleic acid and linoleic acid

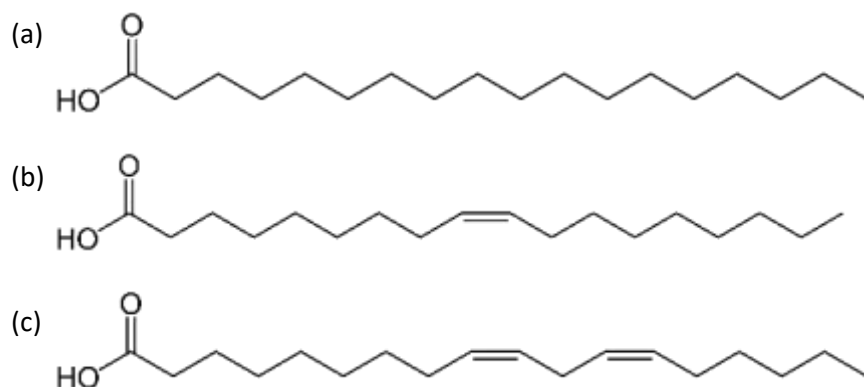
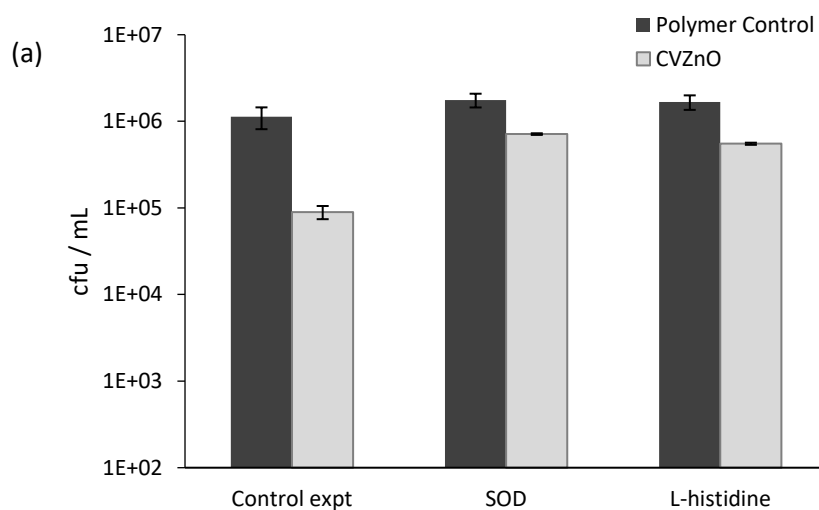


Fig. S12 Structures of (a) stearic acid, (b) oleic acid and (c) linoleic acid. The protonated structures are shown but are in the deprotonated anionic form when bound to Zn^{2+} on the surface of the NPs.

2.10 Hydrogen peroxide and singlet oxygen detection



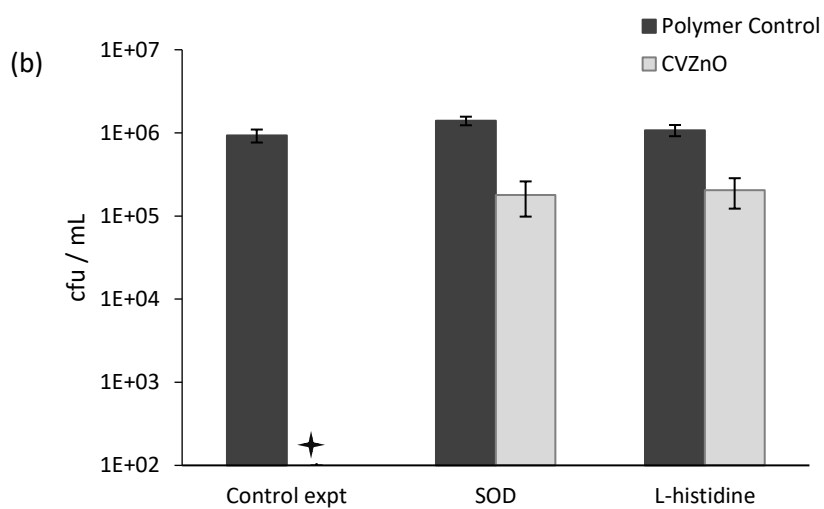
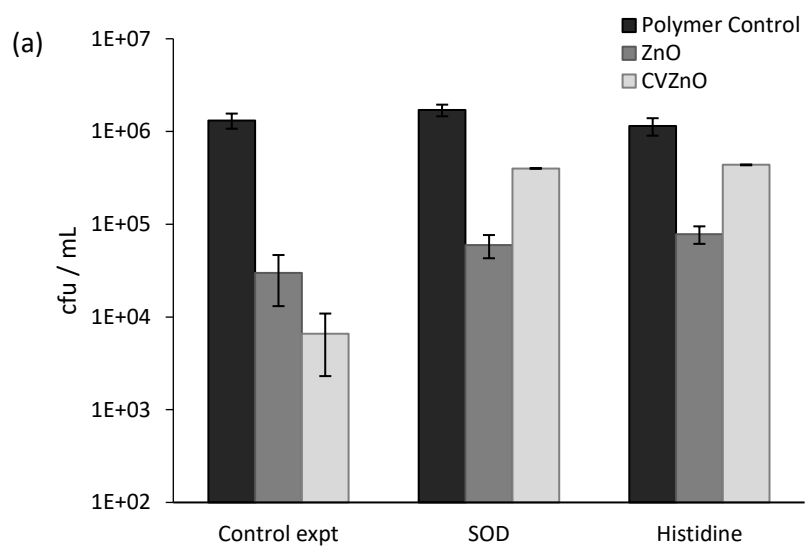


Fig. S13 Viable counts of *S. aureus* NCTC 13143 after (a) 2 h in the dark and (b) 2 h of exposure to white light (500 ± 300 lux) on modified polyurethane samples for a control, superoxide dismutase (SOD) and L-histidine experiment. ♦ indicates bacterial counts were reduced to below the detection limit of 100 cfu/mL.



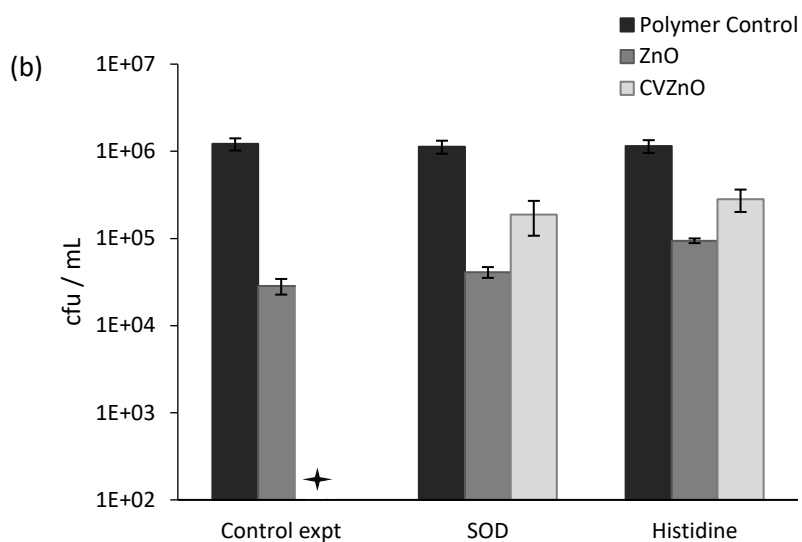


Fig. S14 Viable counts of *E. coli* ATCC 25922 after (a) 2 h in the dark and (b) 2 h of exposure to white light (500 ± 300 lux) on modified polyurethane samples for a control, superoxide dismutase (SOD) and L-histidine experiment. ♦ indicates bacterial counts were reduced to below the detection limit of 100 cfu/mL.

Table. S2 UV-vis absorbance values at 222 nm of furfuryl alcohol after 3 h exposure to standard laboratory white light (500 ± 300 lux) on modified polyurethane samples.

Polymer	Absorbance (A)
Control	0.158
ZnO	0.153
CV	0.138
CVZnO	0.039

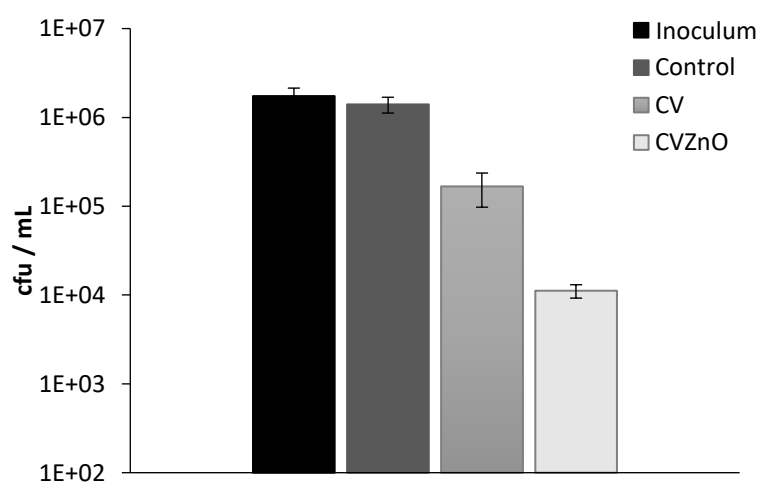


Fig. S15 Viable counts of *E. coli* ATCC 25922 after 4 h exposure to white light (500 ± 300 lux) on modified polyurethane samples with the addition of L-ascorbic acid (1 mM).

References

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