Metal oxide-doped elastomeric materials for amplifying visible light-based antimicrobial activity

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Abstract

Healthcare-associated infection through transmission of pathogenic bacteria poses a huge threat to public health. One of the main transmission routes is via contaminated surfaces, including those of medical devices, and therefore significant efforts are being invested in developing new surface decontamination strategies. This includes visible light-based approaches, which offer improved compatibility with mammalian cells but lower germicidal efficacy with respect to UV-light. This study investigates the potential to enhance the antimicrobial efficacy of 405 nm light for surface decontamination through use of a photocatalytic TiO₂-doped elastomer, elastomers being selected due to their wide use in biomaterials. Poly(dimethylsiloxane) (PDMS) was doped with TiO₂ nanoparticles and the surface elastomer etched to expose the embedded nanoparticles. As etching results in increased surface roughness, samples with control nanoparticles (SiO₂ and Fe₃O₄) were also investigated to decouple the effects of roughness and photoinactivation upon bacterial attachment and inactivation. Characterisation by SEM, AFM and contact angle analysis confirmed that etching produced a rougher (39.3±15.3 vs 5.11±1.29 nm RMS roughness; etched vs unetched TiO₂-PDMS), more hydrophobic surface (water contact angle of 120±2.5° vs 110±1.0°; etched TiO₂-PDMS vs native PDMS). This surface, rich in exposed photocatalytic TiO₂ nanoparticles, allows direct contact between contaminating bacteria and nanoparticles, enabling ROS generation in closer proximity to the bacteria and consequent enhancement of visible light treatment. Incorporating TiO₂ into PDMS significantly improved the photoinactivation efficacy (mean bacterial count for light-treated samples normalised to untreated samples of 0.043±0.0081) compared to PDMS alone (0.19±0.036), when seeded with Staphylococcus aureus and exposed to 405 nm, 60 J cm⁻² light. However, photoinactivation efficacy was significantly (p<0.001) enhanced by etching the TiO₂-PDMS surface (0.015±0.0074), resulting in greater photoinactivation than that obtained for etched (47.0±14.5 nm RMS roughness), non-photocatalytic SiO₂-PDMS (0.10±0.093). Results suggest this doping and etching strategy shows significant potential for facilitating decontamination of elastomer-based biomaterials.
1. Introduction

The transmission of pathogenic bacteria in healthcare settings poses a huge threat to public health with an estimated 3.8 million people acquiring a healthcare-associated infection (HAI) every year in acute care hospitals in Europe, contributing to an estimated 90,000 deaths [1]. One of the main routes of transmission is via contaminated surfaces, including the surfaces of medical devices such as endotracheal tubes, intravenous lines and urinary catheters following invasive surgical procedures [2]. With an ever-increasing number of bacteria becoming resistant to all available antibiotics [3], novel strategies for inhibiting the spread and proliferation of pathogens are urgently required to prevent infections from occurring.

Ultraviolet (UV) light-based decontamination strategies, particularly in the UVC wavelength range, demonstrate strong antibacterial activity [4-6]. However, the use of UV-based treatments for clinical applications is limited by the serious harm UV wavelengths pose to human tissues, as well as the potential for photodegradation of polymeric materials [7].

Visible light-based decontamination approaches do not suffer from these drawbacks and offer an alternative approach to antimicrobial surface treatment. In particular, approaches exploiting the shorter violet-blue wavelengths have shown successful broad-spectrum photoinactivation of microbial species in a range of scenarios, including on surfaces, in solution phase and in biofilms [8-11]. Additionally, the antimicrobial effects of these wavelengths can be exerted at levels that are compatible for mammalian tissue exposure, highlighting the potential for the development of in situ treatments [12-13]. Unlike UVC inactivation, which induces DNA and RNA-based mutations rendering the exposed microbes non-viable [4], violet-blue light wavelengths exert their damage through a photodynamic inactivation process, with porphyrin molecules within the microbial cells acting as endogenous photosensitizers. Absorption of violet-blue light photons by these photosensitive molecules leads to their photoexcitation and subsequent production of a range of reactive oxygen species (ROS) within the cell, including singlet oxygen (\( ^1O_2 \)), superoxide anion radicals (\( O_2^- \)), hydrogen peroxide (\( H_2O_2 \)) and hydroxyl radicals (\( OH^- \)). These reactive species induce non-specific phototoxic effects within the exposed cells, including oxidative damage to proteins, DNA, and lipids that ultimately lead to cell death [14-16].

Despite its broad antimicrobial efficacy and improved compatibility with mammalian cells, violet-blue light is less germicidally efficient than UV light. Photosensitisers can be exploited to photocatalytically enhance the antimicrobial effects of visible light treatments, decreasing the light dose required for effective microbial inactivation. Titanium dioxide (TiO2), an environmentally benign and highly stable material, has been studied extensively as a photocatalyst [17-18] and although its use with UV light is well-documented, its excitation wavelengths extend beyond UV into the visible wavelength region, and the use of visible light for TiO2 photoactivation has shown promising germicidal effects [17,19-21]. The photocatalytic inactivation process involves the absorption of photons and subsequent photoexcitation of the TiO2, which leads to the formation of ROS (primarily hydroxyl radicals) at the TiO2 surface that can degrade organic matter, including bacterial cells through the oxidation of cellular membranes [17]. The action of TiO2-induced ROS on the outer surface of the bacterial cell, combined with the endogenously-produced ROS within the cell during violet-blue light exposure, has the potential to produce an enhanced inactivation process.

The combination of violet-blue light treatment and photosensitisers for surface decontamination has the potential for wide use in healthcare applications. The biocompatibility and stability of TiO2 allows for its incorporation into a range of polymeric materials for medical devices, including commonly used elastomers [22-23] such as polydimethylsiloxane (PDMS). It can be incorporated into materials by a range of different methods [17] that either result in surface coating or bulk doping. Surface coating based approaches have included silver-doped TiO2-PDMS coatings that inhibited Staphylococcus aureus and Staphylococcus epidermidis growth [24] and TiO2 coatings deposited onto PDMS via atomic layer deposition for the inactivation of the yeast Candida albicans in response to UV exposure [25].

The incorporation of TiO2 nanoparticles into a bulk polymer matrix has also been explored. Liu et al [26] recently reported on a TiO2-PDMS matrix capable of inhibiting growth of Escherichia coli when activated by UV light, whilst Correa et al [27] exploited a TiO2-PDMS composite to inactivate a series of microorganisms, and Alberti et al [28] developed a combined sol gel-electrospinning approach to produce PDMS fibres with dispersed TiO2. It is generally accepted that photocatalytic reactions occur principally on the surface of the photocatalyst [29]. However, when bulk doping polymers, the nanoparticles become embedded within the polymer matrix. Etching back the surface matrix can expose the underlying nanoparticles [30], simultaneously producing a roughened surface with high surface area (favourable to photocatalysis). This would allow for closer contact between the photoactive
TiO$_2$ and attached bacteria, potentially increasing cytotoxic ROS exposure. The altered surface roughness would also be expected to further reduce bacterial adhesion and viability [31]. This doping and etching approach to creating TiO$_2$-based antimicrobial materials has not yet been explored in the literature, nor has the amplification of microbial visible light photoinactivation via TiO$_2$-doped polymers.

The present proof-of-concept study uses a 405 nm violet-blue light source to investigate the photoinactivation of bacterial contamination on nanoparticle-doped elastomer surfaces, using the model bacterium *Staphylococcus aureus*, a key causative agent of medical device infections. TiO$_2$ or control Fe$_3$O$_4$ or SiO$_2$ nanoparticles – used to aid in understanding of the effect of surface roughness (these materials having low/negligible photocatalytic activity respectively [32-34]) – were incorporated into a PDMS matrix before etching back the surface polymer to expose the nanoparticles beneath. The resulting surfaces were characterised by scanning electron microscopy (SEM), atomic force microscopy (AFM), and water contact angle analysis.

The efficacy of 405 nm light treatment for inactivation of surface-seeded bacteria was assessed by quantifying the reduction in the viable bacterial populations before and after light exposure, for both etched and unetched materials. The significant enhancement in antibacterial performance observed with the TiO$_2$-doped and etched PDMS surface, together with its ease of manufacture, suggests its suitability for a range of antimicrobial applications in healthcare settings.

2. Materials & Methods

2.1 Materials

The PDMS formulation used was Sylgard™ 184 (Ellsworth Adhesives Europe, UK). The metal oxide nanoparticles used and their properties are outlined in Table 1. The Aeroxide P25 nanoparticles were purchased from Evonik (Germany) and the single-side polished 4” silicon wafers from PI-Kem (UK). All other reagents used for sample preparation were purchased from Sigma-Aldrich (UK).

### Table 1. Nanoparticle Properties

Properties of the nanoparticles used to prepare the metal oxide-doped PDMS, as listed by manufacturer (TiO$_2$ – Evonik, Germany; SiO$_2$ and Fe$_3$O$_4$ – Sigma Aldrich, UK), and naming conventions for the PDMS-based materials produced, alongside images showing the material appearance (8 mm diameter samples).

<table>
<thead>
<tr>
<th>Metal Oxide Dopant</th>
<th>Size (nm)</th>
<th>Purity</th>
<th>Density (g/mL)</th>
<th>Naming (E: Etched, NE: Non-Etched)</th>
<th>Material Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO$_2$ (Aeroxide P25)</td>
<td>14-21</td>
<td>97% (80% anatase 20% rutile)</td>
<td>4.26 (tamped density: 0.13)</td>
<td>TiO$_2$-PDMS-E TiO$_2$-PDMS-NE</td>
<td><img src="Image1" alt="Image" /></td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>10-20</td>
<td>99.5%</td>
<td>2.2-2.6 (bulk density: 0.011)</td>
<td>SiO$_2$-PDMS-E SiO$_2$-PDMS-NE</td>
<td><img src="Image2" alt="Image" /></td>
</tr>
<tr>
<td>Fe$_3$O$_4$</td>
<td>50-100</td>
<td>97%</td>
<td>4.8-5.1 g/mL (bulk density: 0.84)</td>
<td>Fe$_3$O$_4$-PDMS-E Fe$_3$O$_4$-PDMS-NE</td>
<td><img src="Image3" alt="Image" /></td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PDMS-E PDMS-NE</td>
<td><img src="Image4" alt="Image" /></td>
</tr>
</tbody>
</table>
2.2 Preparation of the Nanoparticle-Doped PDMS Samples

The PDMS elastomer base and curing agent were mixed thoroughly in a 10:1 ratio (w/w). Where applicable, metal oxide nanoparticles were then added in a particle:PDMS ratio of 1:4 (w/w) and thoroughly mixed. The resulting mixtures were cast onto a polished silicon wafer, which had been coated with a trichloro(1H,1H,2H,2H-perfluorooctyl) silane release layer by vapour deposition, before being placed in a vacuum desiccator for 30 min to remove air bubbles. After a 1 h settling period at room temperature, the PDMS samples were cured overnight at 50°C. After removing the cured elastomer from the wafer, 8 mm diameter test samples were cut using a biopsy punch. The naming convention used for the materials created is as noted in Table 1, alongside images illustrating the material appearance.

2.2 Etching of the Nanoparticle-Doped PDMS Samples

An etchant solution previously used for the wet etching of PDMS [35] was prepared from a 75% (w/w) aqueous solution of tetrabutylammonium fluoride (TBAF), diluting the TBAF etchant in N-methylpyrrolidinone (NMP) to give a 7.5% working solution. Samples were immersed in freshly prepared etchant solution and continually agitated for 2 min, followed by a 30 s NMP wash and a 30 s ethanol wash before air drying. Non-etched samples were cleaned by 10 min ultrasonication in propan-2-ol. All samples were transferred to a 12-well plate, sterilised by exposure to UV light (Steristrom Disinfection Cabinet, DaRo) and used within 24 h of etching/cleaning.

2.3 Surface Characterisation

SEM analysis of uncoated samples was performed at an accelerating voltage of 10 kV using a Hitachi TM-1000 Tabletop system. Root mean square (RMS) surface roughness was measured by AFM using an Asylum Research MFP-3D AFM system (Oxford Instruments, UK). Surfaces were scanned in tapping mode using AC160TS-R3 probes. A minimum of six well-separated areas were scanned (1 μm × 1 μm scan area; 256 points and lines, unless otherwise noted; 0.5 Hz scan rate). Contact angles were determined from a 2 μl deionised water droplet pipetted onto the sample surface that was imaged using a smartphone with a macro lens attachment. Images were analysed using ImageJ version 1.46 with the drop shape analysis plug-in [36], using the EB-ADSA method, to determine the water contact angle for each surface.

2.4 Bacterial Culture and Preparation of Seeded Surfaces

Staphylococcus aureus NCTC 4135 (National Collection of Type Cultures, Collindale, UK) was inoculated in 100 ml nutrient broth (Oxoid, UK) and incubated at 37°C overnight under constant agitation (120 rpm). The culture was centrifuged for 10 min at 4300 rpm, and the resulting bacterial pellet was re-suspended in 100 ml phosphate buffered saline (PBS; Oxoid, UK) and serially diluted to a population of 1×10^7 colony-forming units per millilitre (CFU ml^-1). To prepare bacterially-seeded PDMS samples, 100 μl volumes of this bacterial suspension were pipetted onto the surface of the 8 mm diameter PDMS samples and left at room temperature for 1 h to allow attachment of the bacteria to the polymer surface. Following this, the droplets were removed by pipette, and the samples were aseptically immersed in sterile PBS to ensure only attached bacteria remained on the sample surface. The samples were then exposed to light treatment, as detailed in the following section.

2.5 Assessment of Bacterial Photo-Inactivation

The violet-blue light source used was a 405 nm LED light array (UNO 24; Photonstar Technologies Ltd., UK) with an approximate bandwidth of 14 nm at full-width half-maximum. The array was powered by a XTITANIUM LED driver (Philips, Netherlands), and a cooling fan and heat sink were attached to the LED array for thermal management. The seeded PDMS samples were positioned below the LED array, providing a constant irradiance of 100 mW cm^-2 at the sample surface, measured using a radiant power meter and photodiode detector (Ophir Optics, Germany). The applied dose was calculated as:

\[
Dose \left( J \text{ cm}^{-2} \right) = \text{Irradiance} \left( W \text{ cm}^{-2} \right) \times \text{Exposure time (s)}
\]

In order to determine baseline inactivation kinetics for the 405 nm light inactivation of S. aureus on non-etched, non-doped surfaces, PDMS-NE samples were exposed to increasing doses of light, up to treatment times of 20 min (120 J cm^-2), with control samples exposed to ambient room light. Upon establishment of the baseline kinetics, exposures of up to 90 J cm^-2 were then repeated with etched PDMS (PDMS-E) and also PDMS doped with TiO_2 (non-etched and etched, PDMS-TiO_2-NE, PDMS-TiO_2-E) in order to understand how the addition of TiO_2 and/or surface etching affects the inactivation kinetics. Doped PDMS samples (etched and non-etched), including the SiO_2 and Fe_3O_4 doped controls, were then subjected to a fixed treatment dose of 60 J cm^-2 (10 min at 100 mW cm^-2) in order to compare antimicrobial efficacy of the differing doping/etching combinations.

Post exposure, to recover surviving bacteria, PDMS samples were aseptically transferred into 1 ml PBS, and subject to manual agitation for 10 s, 10 min ultrasonication, and a further 10 s manual agitation. The resultant suspensions were then serially diluted, and 100 μl samples were spread plated onto tryptone soya agar plates (Oxoid Ltd, UK). Plates were incubated at 37°C for 18-20 hours and bacterial colonies...
counted and recorded as CFU ml⁻¹. The same procedure was used to quantify the bacterial counts present on the PDMS surfaces following the 1 h attachment period, in order to determine the level of bacterial seeding prior to light exposure (i.e. 0 min starting population).

2.5 Statistical Analysis

Experimental data is reported as mean values ± standard deviation. Statistical analysis by 2-sample t-test and analysis of variation (ANOVA), at the 95% confidence level, was performed using Minitab v19 software. Further details on the testing methods and parameters are provided alongside individual results.

3. Results & Discussion

A series of metal oxide-doped PDMS materials were prepared by casting the doped-material onto a polished silicon wafer, in order to ensure a consistently smooth starting surface. Test materials were then etched, using a previously reported etchant solution for PDMS [35], to remove surface elastomer and expose the underlying nanoparticles, whilst control samples remained unetched. This method enables straightforward production of functional materials in a manner that can readily be customised (for example altering dopant materials and concentrations) and can be combined with other fabrication techniques, such as replica moulding [30] and extrusion, to produce medical devices.

3.1 Surface Characterisation of Metal Oxide Doped Samples

The materials produced were characterised to assess key material properties that are known to influence bacterial adhesion and proliferation, notably wettability [37] and surface roughness [31]. Firstly, the etched samples were imaged by SEM (Fig. 1). PDMS cast onto a polished silicon wafer results in a material with a highly smooth surface and, from SEM analysis, etching of the undoped PDMS sample (Fig.1a-b) did not noticeably change this; PDMS-E appeared to be uniform with no roughness or surface features visible at the magnifications used. In contrast, both the TiO₂-PDMS-E (Fig.1c-d) and the SiO₂-PDMS-E (Fig.1e-f) showed a highly roughened surface rich in the metal oxide particles, albeit with slight differences in the appearance of the larger aggregates present. The Fe₃O₄-PDMS-E material exhibited a different surface appearance (Fig.1g-h), with a smaller number of surface particles/aggregates present and large, smooth areas of polymeric material apparent. As all materials were doped at a 1:4 particle:PDMS (w/w) ratio, this can be primarily explained by the substantially higher bulk density of the Fe₃O₄ nanoparticles (see Table 1), although magnetite nanoparticle’s propensity to agglomerate may also play a role.

![Figure 1. SEM images of etched samples. Images show the surface characteristics of PDMS-E (a, b), TiO₂-PDMS-E (c, d), SiO₂-PDMS-E (e, f) and Fe₃O₄-PDMS-E (g, h), imaged at x1000 (left-hand column, scale bar 100μm) and x10,000 (right-hand column, scale bar 10μm) magnification. For PDMS-E, as there were no surface features visible at these magnifications, adsorbed surface debris was searched for (the single bright structure in each image) and used to ensure the sample surface was in focus.](Image)

AFM imaging and RMS surface roughness measurements were performed on all etched materials, along with TiO₂-PDMS-NE and PDMS-NE samples, by tapping mode AFM. Representative images of TiO₂-PDMS-E, SiO₂-PDMS-E, Fe₃O₄-PDMS-E and TiO₂-PDMS-NE surfaces can be seen in Fig. 2, with further images comparing TiO₂-PDMS-NE, PDMS-E and PDMS-NE in SI Fig. 1a. In agreement with the SEM images, these confirm the substantial increase in the roughness of the TiO₂-PDMS-E and SiO₂-PDMS-E materials with respect to the unetched materials (TiO₂-PDMS-NE shown). Overlaying the phase signal over the topography (Fig. 2a) shows, for the etched materials, the presence of strong local phase changes at the material surface (e.g. highlighting
regions with substantially different physical properties), presumably indicative of metal oxide particles protruding from the elastomer matrix. These local phase changes are not present in unetched samples. As expected from the SEM results, the Fe$_3$O$_4$-PDMS-E shows both roughened regions (with local phase changes apparent) and smoother regions (with consistent phase). The presence of these two regions can be seen more clearly when scanning a larger area (SI Fig. 1b).

The RMS roughness measurements (n≥6) obtained are summarised in Fig. 3 (with a constant scale comparison of the topographies in Fig. 2b). The largest mean roughness obtained was for SiO$_2$-PDMS-E (47.0 ± 14.5 nm) followed by TiO$_2$-PDMS-E (39.3 ± 15.3 nm) then Fe$_3$O$_4$-PDMS-E (21.1 ± 19.5 nm), the larger variance for the latter stemming from the two distinct surface topographies present. In contrast, the mean roughnesses of PDMS-E (7.70 ± 2.58 nm), TiO$_2$-PDMS-NE (5.11 ± 1.29 nm) and PDMS-NE (0.813 ± 0.444 nm) were notably smaller, with PDMS-NE being significantly smaller than the other two (significances as discussed in the figure legend).

**Figure 2 (next page).** Representative AFM images of etched and unetched samples. (a) 3D topography images with phase contrast overlay (phase, in degrees, according to colour scale bar) for SiO$_2$-PDMS-E (i), TiO$_2$-PDMS-E (ii), Fe$_3$O$_4$-PDMS-E (iii) and TiO$_2$-PDMS-NE (iv). Note the different ranges in the height (z) and phase signal scale bars for these images. (b) The same topographic measurements shown in 2D with a constant range for the height scale (indicated by colour bar) with (i-iv) as in (a).
Figure 3 (left). RMS roughness of etched and unetched metal-oxide doped PDMS. Horizontal lines indicate the mean, whilst the whiskers correspond to ±1 standard deviation. An asterisk between groups indicates that the RMS roughnesses of all materials in one group were significantly different to all materials in the second group when comparing all six materials using Welch’s ANOVA to account for unequal variances (p<0.05, Games-Howell post-hoc test, n≥6). When the six samples were divided into two groups of equal variance (subdividing the left three and right three materials on the graph) and analysed separately (p<0.05, one-way ANOVA with Tukey’s post-hoc test, n≥6) significant differences in roughness were observed between SiO₂-PDMS-E and Fe₃O₄-PDMS-E and between PDMS-E and TiO₂-PDMS-NE and PDMS-NE.
Sample wettability was determined from sessile drop measurements of the water contact angle on all etched, nanoparticle-doped PDMS samples, along with PDMS-NE, and the results are shown in Table 2. Similar to published values [38], the undoped PDMS surface was hydrophobic with a contact angle of 110°. Etching of the PDMS surface did not significantly alter the surface wettability (107°, p=0.413). However, the addition of TiO$_2$ and SiO$_2$ nanoparticles followed by etching did significantly increase surface hydrophobicity for both TiO$_2$-PDMS-E (120°, p=0.001) and SiO$_2$-PDMS-E (148°, p<0.001). Despite the SEM results having confirmed the presence of nanoparticles at the surface resulting in a visible difference in the surface topography, as with the RMS roughness results, Fe$_3$O$_4$-PDMS-E (112°, p=0.744) did not display a significant change in wettability with respect to native PDMS.

### Table 2. Water contact angles for etched substrates

<table>
<thead>
<tr>
<th>Material</th>
<th>SiO$_2$-PDMS-E *</th>
<th>TiO$_2$-PDMS-E *</th>
<th>Fe$_3$O$_4$-PDMS-E</th>
<th>PDMS-E</th>
<th>PDMS-NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Angle</td>
<td>148° (1.0)</td>
<td>120° (2.5)</td>
<td>112° (1.0)</td>
<td>107° (1.0)</td>
<td>110° (1.0)</td>
</tr>
<tr>
<td>Example Image</td>
<td><img src="example1.png" alt="Image" /></td>
<td><img src="example2.png" alt="Image" /></td>
<td><img src="example3.png" alt="Image" /></td>
<td><img src="example4.png" alt="Image" /></td>
<td><img src="example5.png" alt="Image" /></td>
</tr>
</tbody>
</table>

In summary, these results demonstrate that we have produced a series of materials with varying physicochemical properties that will facilitate decoupling of the effects of roughness and wettability from that of photocatalytic activity. We can firstly compare the effect of embedded TiO$_2$ nanoparticles to that of exposed TiO$_2$ nanoparticles on bacterial photo-inactivation. However, as demonstrated by the above results, etching back of the elastomer changes other surface properties, notably increasing surface roughness and hydrophobicity, both of which could impact bacterial attachment and viability. Therefore, roughened materials created by doping with nanoparticles of negligible or low photocatalytic activity were produced for comparison. Compared to TiO$_2$, extensively reported as a strong photocatalyst [17], SiO$_2$ can be considered essentially non-photocatalytic [32] and Fe$_3$O$_4$ has low photocatalytic activity [33]. Of the resulting etched metal-oxide doped elastomers, TiO$_2$-PDMS-E is intermediate between SiO$_2$-PDMS-E and Fe$_3$O$_4$-PDMS-E both in terms of roughness (SiO$_2$-PDMS-E > TiO$_2$-PDMS-E > Fe$_3$O$_4$-PDMS-E) and hydrophobicity (SiO$_2$-PDMS-E >> TiO$_2$-PDMS-E > Fe$_3$O$_4$-PDMS-E), with SiO$_2$-PDMS-E being notably more hydrophobic with a mean water contact angle nearing superhydrophobicity (usually defined as a water contact angle of ≥150°) [39]. Consideration of these combined photocatalytic, roughness and wettability properties will aid in the interpretation of the following bacterial photo-inactivation results.

### 3.2 Violet-Blue-Light Photo-Inactivation of *S. aureus* on Metal Oxide-Doped Samples

Exposure of *S. aureus* to 405 nm light on non-etched, non-doped PDMS was first characterised in order to establish baseline 405 nm light inactivation kinetics, and also to identify treatment doses for use in the study where photocatalytic enhancement could be observed (SI, Fig. 2 and PDMS-NE data points in Fig. 4). *S. aureus* contamination was significantly reduced (p<0.05) compared to non-exposed controls, with reductions of 0.61, 0.73 and 1.99 log$_{10}$ CFU ml$^{-1}$ following exposure to 30, 60 and 90 J cm$^{-2}$, respectively. The level of *S. aureus* contamination on the non-etched PDMS-NE over a 20 min treatment time showed no significant change (p=0.186; one-way ANOVA), demonstrating that inactivation was due to the light treatment (see SI, Fig. 2).

The comparative efficacy of 405 nm light for decontamination of etched PDMS (PDMS-E) and of TiO$_2$-doped surfaces, with and without etching (TiO$_2$-PDMS-E and TiO$_2$-PDMS-NE, respectively), was then investigated using the same treatment doses (up to 90 J cm$^{-2}$) (Fig. 4). The TiO$_2$ nanoparticle formulation used was 80% anatase; anatase being the most potent form of TiO$_2$ for ROS production [17]. There was no significant difference (p<0.05, one-way Welch’s ANOVA) between materials in the number of surviving bacteria recovered from unexposed samples. However, at doses of both 30 and 60 J cm$^{-2}$ there was a clear increase in efficacy of *S. aureus* inactivation upon TiO$_2$ doping and etching. At 60 J cm$^{-2}$, TiO$_2$-PDMS-E showed a significant improvement in inactivation over the undoped materials, with a mean log$_{10}$ survival count of 3.25 CFU ml$^{-1}$ compared to 3.72 for PDMS-E (p=0.045) and 4.07 for PDMS-NE (p<0.001). This suggests that some ROS diffusion from nanoparticles immediately below the surface, through the PDMS matrix, towards the surface-adsorbed bacteria is occurring [17]. However, a much greater improvement was obtained upon
etching of the material, with TiO$_2$-PDMS-E showing a significantly (p=0.002) lower survival rate (2.39 log$_{10}$ CFU ml$^{-1}$) than when unetched. This strong enhancement in bacterial inactivation obtained by doping and etching was also observed after exposure to only 30 J cm$^{-2}$, where TiO$_2$-PDMS-E showed a significantly lower survival rate (3.30 log$_{10}$ CFU ml$^{-1}$) than all other materials (3.90/p=0.005, 4.25/p=0.001 and 4.47/p=0.001 for TiO$_2$-PDMS-E, PDMS-NE and PDMS-E respectively).

In order to further examine the inactivation enhancement obtained with the TiO$_2$-doped PDMS, further testing was conducted using a fixed dose of 60 J cm$^{-2}$. This dose was selected as it was the exposure level which demonstrated significant differences between the inactivation achieved on the etched and on the nonetched TiO$_2$-doped surfaces as compared to the undoped materials, p=0.05 (as indicated by statistical analysis of the samples exposed to 60 J cm$^{-2}$, shown in Fig. 4). SiO$_2$ and Fe$_3$O$_4$ doped substrates were included in order to aid in decoupling the effects of surface roughness from photocatalysis of TiO$_2$ nanoparticles. However, as etching has a pronounced effect on the surface roughness and wettability (as demonstrated above), in order to make surface-to-surface comparisons, it was important to first consider the level of initial bacterial attachment on each surface before light exposure. Fig. 5 shows that bacterial attachment on all non-etched surfaces was comparable with no statistical difference between the materials (when the four unetched materials, for which the log$_{10}$ bacterial counts were all within the range 4.9 – 5.0 CFU ml$^{-1}$, were compared in a one-way ANOVA, no difference was observed, p=0.726). Similarly there was no statistically significant difference in attachment on PDMS-E vs PDMS-NE (p=0.163, 2-sample t-test).

However, attachment on etched, doped-surfaces, was found to be altered, with a significant reduction (p=0.05; 2-sample t test) in the level of attached bacteria for etched materials in comparison to unetched (e.g. comparing pairs of materials with the same dopant). Notably, SiO$_2$-PDMS-E surfaces had the lowest level of bacterial attachment, with 36.3% of the level of that on PDMS-NE (a significant reduction, p<0.001 by one-way ANOVA with Dunnett’s post-hoc test; corresponds to a mean bacterial count of 4.54 log$_{10}$ CFU ml$^{-1}$). Given that increasing surface roughness is known to decrease bacterial adhesion [31, 40], as is increasing surface hydrophobicity [37], this result could be anticipated from SiO$_2$-PDMS-E having been shown to have significantly higher RMS roughness and water contact angle than all other materials. Etching also significantly reduced attachment on Fe$_3$O$_4$-doped PDMS, which had 60.8% of the bacterial count on PDMS-NE (p=0.029; corresponds to a mean bacterial count of 4.79 log$_{10}$ CFU ml$^{-1}$), and to a lesser extent on TiO$_2$-doped PDMS (a non-significant, p=0.120, reduction of 69.0% of the bacterial count on PDMS-NE; corresponds to a mean

**Figure 4.** 405 nm light inactivation of *S. aureus* on doped and etched PDMS substrates. Inactivation of bacteria seeded onto non-etched and etched PDMS surfaces (PDMS-NE, PDMS-E, respectively) compared to that on non-etched and etched TiO$_2$-doped PDMS (TiO$_2$-PDMS-NE, TiO$_2$-PDMS-E, respectively) with increasing doses of 405 nm light at an irradiance of 100 mW cm$^{-2}$. Data represents the level of surviving bacteria as the mean log$_{10}$ CFU ml$^{-1}$ (n=6±SD) for each condition. * indicates a significant difference in survival rate from all other materials at that dose (p<0.05, one-way Welch’s ANOVA with Games-Howell post-hoc test).

**Figure 5.** Bacterial attachment on non-etched and etched PDMS, TiO$_2$-PDMS, SiO$_2$-PDMS and Fe$_3$O$_4$-PDMS surfaces. Levels of *S. aureus* recovered from the surfaces following a 1 h attachment period (n=6±SD), with mean counts (measured as CFU ml$^{-1}$) normalised to those of PDMS-NE. * represents significant difference in attachment between NE and E surfaces (p<0.05; 2-sample t test). # represents a significant difference compared to PDMS-NE (p<0.05; one-way ANOVA with Dunnett’s post-hoc test).
bacterial count of 4.85 log10 CFU ml⁻¹. As the characterised physicochemical properties of the TiO₂-PDMS-E surface were more similar to the properties of SiO₂-PDMS-E than that of Fe₂O₃-PDMS-E, this result was slightly surprising and may possibly be due to the highly heterogenous surface of the Fe₂O₃-PDMS-E or to additional surface properties as yet uncharacterised.

405 nm visible light-induced inactivation was then assessed for all eight materials (at 60 J cm⁻²) alongside control samples for each, which were exposed only to ambient room light (for the same exposure period). All surfaces showed a significant reduction in bacterial contamination on the 405 nm light exposed surface, compared to the respective control sample (p<0.05, 2-sample t-tests). Fig. 6 presents the inactivation data as normalised bacterial counts, normalising the mean counts of surviving bacteria (CFU ml⁻¹) on the 405 nm exposed samples to that of the control samples. This takes into account the aforementioned differences in S. aureus attachment across the materials (which results in different starting bacterial populations), as well as any inactivation that occurs during the exposure period that is not a direct consequence of 405 nm light exposure e.g. it shows inactivation due to 405 nm light exposure only.

![Figure 6. 405 nm light inactivation of S. aureus on non-etched and etched PDMS, TiO₂-PDMS, SiO₂-PDMS and Fe₂O₃-PDMS surfaces.](image)

This greatest enhancement for TiO₂-PDMS-E in normalised, 405 nm light-induced bacterial inactivation confirms that the exposure of TiO₂ nanoparticles produced by surface etching results in a substantial enhancement of photocatalytic activity. No difference from PDMS-NE was observed for SiO₂-PDMS-E (p=0.455), suggesting that the enhanced bacterial inactivation of TiO₂-PDMS-E was not a consequence of increased surface roughness or hydrophobicity but was due to the exposure of the embedded photocatalytic particles.

4. Conclusion

We have shown that a combined doping and etching strategy produces an elastomeric material with a surface rich in photocatalytic TiO₂ nanoparticles, enabling direct contact of attached bacteria with the TiO₂, which would be expected to result in ROS generation in close proximity to adsorbed bacteria. We have demonstrated that the resulting material is effective in amplifying visible light-induced bacterial inactivation, even at low doses (e.g. 30 J cm⁻²). Our results also highlighted the reduced bacterial attachment can result from the highly roughened surfaces produced by nanoparticle doping and etching back of the elastomer, particularly in the case of SiO₂ doping. Future research efforts will focus on combining these two properties into a single material, employing different combinations of dopants to create a surface that reduces the ability of microbes to adhere to elastomeric materials and that, for those microbes that do adhere, the surface is rich in exposed photocatalytic sites for effective photoinactivation. Investigation of the influence of irradianc levels and treatment times on the photocatalytic antibacterial efficacy of these surfaces will also be important to establish the efficacy of clinically-relevant treatment conditions on germicidal activity and to create a highly robust strategy for the decontamination of elastomeric surfaces.

5. Supplementary Material
Two supplementary results figures are contained within the Supplementary Material document.

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8. Data statement

All data underpinning this publication are openly available from the University of Strathclyde KnowledgeBase at: https://doi.org/10.15129/a419251e}

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