
Access from the University of Nottingham repository:
http://eprints.nottingham.ac.uk/52259/8/1-s2.0-S0264127518304453-main.pdf

Copyright and reuse:

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the Creative Commons Attribution licence and may be reused according to the conditions of the licence. For more details see: http://creativecommons.org/licenses/by/2.5/

A note on versions:

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact eprints@nottingham.ac.uk
Generation and characterisation of gallium titanate surfaces through hydrothermal ion-exchange processes

Matthew D. Wadge *, Bryan W. Stuart, Kathryn G. Thomas, David M. Grant *

Advanced Materials Research Group, Faculty of Engineering, University of Nottingham, UK

**HIGHLIGHTS**

• Gallium (9.4 at.%) can successfully ion-exchange with sodium (2.7 at.%) in titanate structures (0.5–1 μm deep).
• RHEED analysis was successfully conducted, for the first time, confirming d spacing values for titanate structures.
• Pre-heat-treated gallium titanate (2.76 ppm) released more gallium ions compared to post-heat-treated samples (0.68 ppm).
• Released gallium ion concentrations (4–40 μM) were significantly less than toxic concentrations for S. aureus (0.3–5.1 mM).
• Gallium titanate showed significant (p<0.0001) cytotoxicity (76% cell viability reduction) vs. heat-treated layers (19% reduction).

**GRAPHICAL ABSTRACT**

**ABSTRACT**

Infection negation and biofilm prevention are necessary developments needed for implant materials. Furthermore, an increase in publications regarding gallium (Ga) as an antimicrobial ion has resulted in bacterial-inhibitory surfaces incorporating gallium as opposed to silver (Ag). The authors present the production of novel gallium titanate surfaces through hydrothermal ion-exchange reactions. Commercially-pure Ti (50: Cp-Ti) was initially suspended in NaOH solutions to obtain sodium titanate (S1: Na2TiO3) layers ca. 0.5–1 μm in depth (2.4 at.% Na). Subsequent suspension in Ga(NO3)3 (S2: Ga2(TiO3)3), and post-heat-treatment at 700 °C (S3: Ga2(TiO3)3-HT), generated gallium titanate layers (9.4 and 4.1 at.% Ga, respectively). For the first time, RHEED analysis of gallium titanate layers was conducted and demonstrated titanate formation. Degradation studies in DMEM showed S2: Ga2(TiO3)3 released more Ga compared to S3: Ga2(TiO3)3-HT (2.76 vs. 0.68 ppm) over 168 h. Furthermore, deposition of Ca/P in a Ca:P ratio of 1.71 and 1.34, on S2: Ga2(TiO3)3 and S3: Ga2(TiO3)3-HT, respectively, over 168 h was seen. However, the study failed to replicate the antimicrobial effect presented by Yamaguchi who utilised A. baumannii, compared to S. aureus used presently. The authors feel a full antimicrobial study is required to assess gallium titanate as a candidate antimicrobial surface.

© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

**KEYWORDS:** Biomaterial, Sodium titanate, Gallium titanate, Hydrothermal, Ion-exchange, Titanium

**1. Introduction**

The extent of a medical implant’s success in vivo is dependent upon growth of extracellular tissue up to, and around, the implant via...
osteoconduction and osteogenesis [1]. In recent years, significant emphasis has been directed towards improving adhesion between implant surfaces and local tissues through direct surface modifications [2–4].

The only FDA approved process for improving implant surfaces utilises high-temperature (droplet temperatures >1500 K [5]) plasma spray methods to deposit coatings of osteoconductive hydroxyapatite (HA) [6]; mimicking the main mineral component, and chemical and crystal structure, of cortical bone. These coatings, therefore, are ideal for improving metallic implant biocompatibility and enhancing osseointegration [7]. However, current plasma-spraying techniques offer poor adhesion [8]; non-uniformity in coating density [9]; excessive temperatures leading to deleterious phase transformations [10]; as well as residual surface stresses [11] resulting in micro-crack formation [12]. Ultimately, plasma-sprayed HA layers have been shown to spall due to their brittle nature [13], and weak mechanical adhesion (55–62 MPa; just higher than the FDA’s minimum requirement of 50.8 MPa) [14,15]. Spalled particles may embed within surrounding tissue, activating complex cellular pathogenesis networks, fundamentally leading to potentially high manufacturing costs [20].

Therapeutic methods for providing a stable HA layer have been proposed, [14,15]. Spalled particles may embed within surrounding tissue, activating complex cellular pathogenesis networks, fundamentally leading to potentially high manufacturing costs [20].

To overcome these limitations, solution-based surface treatments have been considered [21–23], including the production of sodium titanate surfaces [24]. Research by Kokubo et al. [25–32] identified the formation of sodium titanate through hydrothermal synthesis, therefore, preventing coating spallation caused by excessive production temperatures. Studies confirmed that optimal surface formation occurred at 60 °C, much lower than current plasma-spraying technologies. Once generated, and following further heat- and water-treatments, Ca and P ion-exchanges with the Na modifier within the sodium titanate structure occur. This allows HA generation upon implantation in vivo or submersion in simulated body fluid (SBF) in vitro, offering an attractive processing methodology [28].

Failure of implants still persists as a substantial issue in orthopaedic hip replacements, with most common factors including infection (25–28%), and mechanical loosening (19%) [33,34]. Implant infection is a complex issue as bacteria entering the surgical site adhere to implant surfaces and form a ‘biofilm’, protecting individual bacteria from antibiotics and the patient’s immune system [35]. Initial prevention of biofilm formation is an attractive solution [36]. One possible method for biofilm prevention is the utilisation of antimicrobial ions, such as copper (Cu), silver (Ag), and more recently, gallium (Ga) [37,38].

Despite its prevalence, the use of Ag has been extensively debated in medical devices [39]. This is because there are conflicting results in the literature, for example various in vitro studies have demonstrated cytotoxic effects on host fibroblasts and keratinocytes [40,41], whilst others have shown minimal, to no, sequelae in vivo [42]. A review by Brett demonstrated the majority of in vivo studies indicate silver’s non-cytotoxicity, however, its ability to bind to proteins and nucleic acids may result in higher topical dosages being needed to generate antimicrobial effects [39]. Furthermore, studies have shown Ag’s limited capacity to fully protect against infections, which has resulted in increased concern for its use in medical devices [43].

Ga^{3+} ions have been purported to be an ideal substitute for Ag in antimicrobial surfaces through various anti-bacterial studies [44,45]. Their similarity to Fe^{3+} in ionic radius and charge, allow replacement within target molecules, which has resulted in an ideal antimicrobial agent, whose presence can cause Ga-induced bacterial metabolic distress [44,46]. A further property, which is pertinent to orthopaedic applications, is the inhibition of bone resorption through reduction in calcium release from bone [47]. Therefore, in this work, the authors present extensive characterisation of gallium titanate surfaces produced through ion-exchange reactions of sodium titanate produced via hydrothermal synthesis. In addition to cross-section electron microscopy, RHEED analysis on the top few nm of the titanate’s surface, in conjunction with XPS of the same surface, to elucidate their structure and chemistry, is presented. Additionally, a pilot study to assess the cytotoxicity and antimicrobial nature of these surfaces is shown.

The antimicrobial nature of gallium titanate surfaces has been assessed previously by Yamaguchi et al. using a nosocomial, multidrug resistant, Gram-negative bacteria: A. baumannii [48], although using a different processing route. However, assessment using a Gram-positive bacteria of gallium titanate surfaces has yet to be investigated, hence the conducted pilot study using S. aureus (Newman). This is presented here along with the detailed characterisation and stability of using different hydrothermal conditions and concentrations compared to Yamaguchi, and its stability in media, pre- and post-heat-treatment, to fully understand the potential of this route.

Ion-exchange routes in low temperature solutions (60 °C) have the potential to enable low cost and scalable generation of osteogenic, antimicrobial surfaces, in comparison to plasma-spraying and physical vapour deposition [28,30]. Another key advantage is its ability to manipulate surface chemistry reactions and utilise the ion-exchangeability of Na2TiO3 with ions including Ca, P, Mg, Ga, and Ag. This will enable further tailoring and design of surfaces, which could combine a customised array of therapeutic ions to treat individual requirements; a stratified approach to design [49–52]. Furthermore, solution-based methodologies encourage sufficient penetration into porous morphologies to facilitate cellular infiltration, which is limited with conventional line of site coating methods [49].

2. Methodology

2.1. Substrate preparation

Commercially-pure Ti (Grade 1) discs (10 mm ∅, 1 mm thick), herein labelled as S0: Cp-Ti, were used as substrates. Discs were ground and polished using varying grits (P280, P400, P800, P1200, P2500 and P4000) of silicon carbide paper. The discs were cleaned by sonicating in acetone followed by distilled water for 5 min each.

2.2. Sodium hydroxide hydrothermal treatment

A 5 M solution of NaOH was prepared by dissolving 19.99 g of NaOH pellets (purity: 99.0%, Sigma-Aldrich) in 100 mL of distilled water. 10 mL aliquots in triplicate were then heated in water baths and individual S0: Cp-Ti substrates were placed in each polypropylene container at 60 °C for 24 h. Na-exchanged samples were labelled as S1: Na2TiO3.

2.3. Ion-exchange treatments

Gallium ion-exchange reactions were conducted from S1: Na2TiO3, using a 4 mM solution of Ga(NO3)3. The solution was prepared by dissolving 0.1 g of Ga(NO3)3·xH2O granules (x = 1–9) (purity: 99.9%, Sigma-Aldrich) into 100 mL of water. 10 mL aliquots in polypropylene containers were heated at 60 °C in water baths for 24 h. Ga-exchanged titanate samples have been labelled S2: Ga2(TiO3)3.

2.4. Heat-treatments

Both S0: Cp-Ti and S2: Ga2(TiO3)3 were heat-treated to produce S4: Cp-Ti-HT and S3: Ga2(TiO3)3-HT, respectively, using a Lenton® furnace in air with a ramp rate of 5 °C min⁻¹ to 700 °C. All samples were left to dwell for 1 h followed by natural furnace cooling to room temperature.

2.5. Scanning electron microscopy (SEM)

Micrographs were obtained by Scanning Electron Microscopy (SEM) via a JEOL 6490LV SEM. A constant working distance of 10 mm was
Fig. 1. (A, C, E, G, and I) FEG-SEM surface and (B, D, F, H, and J) cross-sectional images of S0: Cp-Ti, S1: Na2TiO3, S2: Ga2(TiO3)3, S3: Ga2(TiO3)3-HT, and S4: Cp-Ti-HT samples, respectively. Inset images are of the corresponding sample’s surface.
maintained, utilising a beam energy of 15 kV. Image acquisitions for higher resolution scans were conducted on a Field-Emission Gun Scanning Electron Microscope (JEOL 7100 FEG-SEM).

2.6. Energy dispersive X-ray spectroscopy (EDX)

Surface compositional analysis was determined via an Energy-Dispersive X-ray spectrometer (EDX) (Oxford Instruments) at a working distance of 10 mm, a beam voltage of 15 kV, and maintaining a minimum X-ray count of 150,000 counts.

2.7. X-ray diffraction (XRD)

Crystallinity was assessed using a Bruker D8 advanced XRD spectrometer (Cu Kα source, λ = 1.5406 Å, 40 kV, 35 mA). Measurements were taken over a 2θ range from 25 to 65°; with a step size of 0.04° (2θ); a glancing angle of 2°; and a dwell time of 12 s. The glancing angle allows the X-ray beam to graze the surface, penetrating the first few microns of material, and restricting the diffraction signal to the same depth [53].

2.8. Reflective high-energy electron diffraction (RHEED)

Shallow angle diffraction analysis was conducted using a JEOL 2000 FX TEM with an attached RHEED stage and photographic plate camera. Film acquisition was obtained using an accelerating voltage of 200 kV, and an exposure time between 11 and 22 s to ensure visible diffraction rings were present. Diffraction ring radii were then analysed using an image processing software and appropriate d-spacing values were calculated according to Bragg's law. Calibration was conducted using a sputtered gold layer on the surface of an S0: Cp-Ti substrate.

2.9. Raman spectroscopy

Raman spectroscopy was achieved utilising a HORIBA Jobin Yvon LabRAM HR spectrometer. Spectra were acquired using a 532 nm laser (25 mW power), 50× objective, and a 300 μm confocal pinhole. For simultaneous scanning of multiple Raman shifts, a 600 lines/mm rotatable diffraction grating along a path length of 800 mm was used. Detection of spectra was achieved through the use of a SYNAPSE CCD detector (1024 pixels) thermoelectrically cooled to −60 °C. Instrument calibration using the Rayleigh line at 0 cm⁻¹ and a standard Si (100) reference band at 520.7 cm⁻¹, was employed prior to spectra acquisition. A constrained time window of 20 s was employed for each spectra recording with 20 accumulations.

2.10. Fourier transform infrared spectroscopy (FTIR)

Infrared absorbance was surveyed using a Bruker Tensor FTIR spectrometer with an Attenuated Total Reflectance (ATR) attachment containing a diamond crystal/ZnSe lens. λ of 2.5 to 20 μm were surveyed, corresponding to 4000 and 500 cm⁻¹, respectively.

2.11. X-ray photoelectron spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) was conducted using a VG ESCALab Mark II X-ray photoelectron spectrometer with a monochromatic Al Kα X-ray source incident to the sample surface at ≈30°. Survey and high-resolution scans were conducted in addition to the measurement of adventitious C 1s for calibration: charge corrected to 284.8 eV. Parameters for acquisition were as follows: step size of 1.0; number of scans set at 5; dwell time 0.2 s for survey scans, and 0.4 s for high-resolution scans. Binding energies were measured over a range of 0–1200 eV. All spectra were analysed in Casa XPS constraining the Full Width at Half Maximum to the same value for all deconvoluted spectral peaks for the same element.

2.12. Ion leaching via induction coupled plasma (ICP)

Samples were degraded in 1 mL DMEM and were removed after varying degradation times of 6 h, 24 h, 3 days (72 h), and 7 days (168 h). During removal, the samples were washed with 9 mL of ultrapure water, ensuring a serum dilution of 1:10, before being removed and subsequently washed in ultrapure water and air dried. The 10 mL solutions were then analysed using inductively coupled plasma mass spectrometry (ICPMS; Thermo-Fisher Scientific ICAP-Q with CCTED). Each time point had three samples independently prepared, with calculated standard error and mean values presented.

2.13. Neutral red uptake (NRU) assay

Samples were degraded in 1 mL DMEM containing Fetal Bovine Serum for 7 days at 37 °C, generating liquid extracts as described in ISO 10993-5:2009. The extended degradation time was used to mimic long-term contact with the body. MG-63 cells were seeded into a 24 well plate (20,000 cells cm⁻²) and incubated for 24 h to give a sub-confluent monolayer. The media was removed and replaced with the liquid extracts. After 24 h further incubation the media was removed, the cells washed with PBS, and 500 μL of Neutral Red medium was added. After 2 h incubation, the medium was removed, cells were washed in PBS, and 500 μL of de-stain was added per well. Plates were shaken on a plate shaker for 10 min and the NR absorption read using an ELx800 Microplate Colorimeter (BioTek Instruments Inc.) at 540 nm.

2.14. LIVE/DEAD assay

S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT samples alongside S0: Cp-Ti controls were sterilised via UVB light (Naure Class II Safety Cabinet) for 30 min per side. S. aureus Newman strain was cultured in Tryptone Soy Broth (TSB) overnight. Samples of each type were added in triplicate to sterile petri dishes and 15 mL pre-warmed (37 °C) TSB added. The overnight culture was washed twice in TSB, and then used to inoculate the petri dishes to 0.01 OD₆₀₀. The dishes were incubated (37 °C at 60 RPM) for 3 days, followed by washing in distilled water twice, then incubated at room temperature in the dark for 30 min with BacLight LIVE/DEAD stain (Invitrogen), and finally dried. The samples were imaged on a Carl Zeiss L700 Confocal Laser Scanning Microscope and biomass volume analysed via COMSTAT 2 plugin in ImageJ [54].

3. Results

3.1. Compositional analysis

3.1.1. SEM

Surface alterations were tracked following each ion-exchange reaction and post-heat-treatment. After NaOH treatment at 60 °C (S1: Na₂TiO₃), some alteration to the morphology of Ti surfaces from S0: Cp-Ti was exhibited (Fig. 1A & C). Extended nano-porous networks were seen. Following Ga ion-exchange, micrographs of S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT samples over a 400 μm² area of the sample surface. Mean atomic percent (at.%)(Mean ± S.E.M.; n = 3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Elemental composition / at.%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
</tr>
<tr>
<td>S0: Cp-Ti</td>
<td>100</td>
</tr>
<tr>
<td>S1: Na₂TiO₃</td>
<td>31.9 ± 0.1</td>
</tr>
<tr>
<td>S2: Ga₂(TiO₃)₃</td>
<td>20.1 ± 0.2</td>
</tr>
<tr>
<td>S3: Ga₂(TiO₃)₃-HT</td>
<td>22.6 ± 0.4</td>
</tr>
<tr>
<td>S4: Cp-Ti-HT</td>
<td>30.2 ± 0.1</td>
</tr>
</tbody>
</table>
showed a similar interconnected morphology to S1: Na₂TiO₃ (Fig. 1E). Upon heat-treatment (S3: Ga₂(TiO₃)₃-HT), a slightly modified interconnected morphology remained, with the formation of flake-like features on the surface, with diameters of 150–300 nm (Fig. 1G). The inclusion of S4: Cp-Ti-HT (Fig. 1I), was to identify morphological differences between sodium titanate and rutile formation on the sample’s surface. The surface of S3: Ga₂(TiO₃)₃-HT was significantly dissimilar to that of S4: Cp-Ti-HT with a porous angular surface containing oblong flakes of ca. 0.5 μm.

Cross-sectional FEG-SEM imaging of S1: Na₂TiO₃, S2: Ga₂(TiO₃)₃, and S3: Ga₂(TiO₃)₃-HT showed similar morphology, with a distinct porous layer of the order of 0.5–1 μm in thickness (Fig. 1D, F, & H). This is in stark contrast to the original smooth S0: Cp-Ti control sample (Fig. 1B). However, the layer exhibited in S3: Ga₂(TiO₃)₃-HT demonstrates an intermediate layer between the nanoporous surface layer and the titanium substrate (Fig. 1H). Furthermore, S4: Cp-Ti-HT demonstrated a different cross-sectional profile to all other samples with a thin, dense titanium oxide layer (Fig. 1J).

Fig. 2. (A, B, C and D) RHEED diffraction patterns for S1: Na₂TiO₃, S2: Ga₂(TiO₃)₃, S3: Ga₂(TiO₃)₃-HT, and S4: Cp-Ti-HT, respectively. (E) XRD data of aforementioned samples. Deconvolution of the peaks are as follows: ▲ - rutile (TiO₂: ICDD PDF 00-021-1276); ▼ - titanium oxide (Ti₆O: ICDD PDF 01-072-1471); – gallium titanate (Ga₂TiO₅: ICDD PDF 00-020-0447); ● - titanium (Ti: ICDD PDF 00-044-1294).

268 M.D. Wadge et al. / Materials and Design 155 (2018) 264–277
3.1.2. EDX

Initially, elemental mapping analysis of S1: Na2TiO3 showed homogeneous distribution of Na, Ti and O, and concluded Na (2.73 at.%) and O (65.3 at.%) had been included within the structure, compared to the S0: Cp-Ti control. Subsequent analysis of S2: Ga2(TiO3)3 indicated complete substitution of Na by Ga within the TiO3 structure. S2: Ga2(TiO3)3 compared to S3: Ga2(TiO3)3-HT showed a 5.3 at.% reduction of Ga within the later following heat-treatment (Table 1).

3.1.3. XRD

As seen in Fig. 2E, the only signals present for S1: Na2TiO3 and S2: Ga2(TiO3)3 were that of the Ti substrate (S0: Cp-Ti), which produced peaks associated with titanium (Ti: ICDD PDF 00-044-1294). Following heat-treatment (S3: Ga2(TiO3)3-HT), further diffraction peaks emerged located at ≈26, 36, 38, and 55° 2θ, which were attributed to gallium titanate (Ga2TiO5: ICDD PDF 00-037-0346), however, the lack of high quality diffraction data for gallium titanate, the lower intensity, as well as the overlap of gallium titanate with rutile means XRD data alone is inconclusive. The peak at ≈57° 2θ correlated to rutile (TiO2: ICDD PDF 00-021-1276), and peaks at ≈37, 40, and 53° 2θ related to titanium oxide (TiO: ICDD PDF 01-072-1471). To verify this further, RHEED analysis was conducted as this technique offers greater probing resolution and shallower probing depth (0.1 μm). The analysis of S4: Cp-Ti-HT revealed bands located at ≈247, 445, and 611 cm⁻¹, which were attributed to rutile, Ti−O. Conversely, alternate peaks were found in the S2: Ga2(TiO3)3 sample at ≈273, 425, 700, and 811 cm⁻¹, as well as ≈400 and 662 cm⁻¹ in S1: Na2TiO3. A shoulder was present in both S3: Ga2(TiO3)3-HT and S4: Cp-Ti-HT at ≈700 cm⁻¹, which was also present as an identifiable peak in S2: Ga2(TiO3)3.

3.1.4. RHEED

RHEED analysis of S4: Cp-Ti-HT (Fig. 2D) demonstrated clear and distinct diffraction rings, as well as matching d spacing values with rutile (TiO2: ICDD PDF 00-021-1276: Table 2) consistent with the SEM-EDX and XRD results. The diffraction patterns present in S1: Na2TiO3, S2: Ga2(TiO3)3, and S3: Ga2(TiO3)3-HT (Fig. 2A, B, and C, respectively) demonstrated a significant change from that of S4: Cp-Ti-HT, indicating an alternative layer to rutile (Fig. 2D). The d spacing values for S1: Na2TiO3 were ascribed to sodium titanate (Na0.23TiO2: ICDD PDF 00-044-1294), sodium titanate (Na4TiO4: ICDD PDF 00-025-1450), and calcium titanate (CaTi2O5: ICDD PDF 00-025-1450), as well as S3: Ga2(TiO3)3-HT being similar to gallium and calcium titanate variants (Ga2TiO5: ICDD PDF 00-021-1276, and CaTi2O5: ICDD PDF 00-025-1450).

3.1.5. Raman

Raman spectral analysis (Fig. 3A) of S3: Ga2(TiO3)3-HT and S4: Cp-Ti-HT revealed bands located at ≈247, 445, and 611 cm⁻¹, which were attributed to rutile, Ti−O. Conversely, alternate peaks were found in the S2: Ga2(TiO3)3 sample at ≈273, 425, 700, and 811 cm⁻¹, as well as ≈400 and 662 cm⁻¹ in S1: Na2TiO3. A shoulder was present in both S3: Ga2(TiO3)3-HT and S4: Cp-Ti-HT at ≈700 cm⁻¹, which was also present as an identifiable peak in S2: Ga2(TiO3)3.

3.1.6. FTIR

IR absorption showed peaks detailed from 500 to 900 cm⁻¹, matching TiO2 vibrations, Ti−O bending and Ti−OH non-bridging bonds, which were prevalent across all samples (Fig. 3B). Additionally, a peak around 1100 cm⁻¹ and a broad peak from 3000 to 3500 cm⁻¹, which appear in S1: Na2TiO3 and S2: Ga2(TiO3)3, correspond to Ti−O−C vibrations and H−O−H stretching, respectively. Three peaks at 1130, 1300, and 2350 cm⁻¹ were seen in the S4: Cp-Ti-HT control, consistent with rutile Ti−O, Ti−O−Ti stretching, and CO2 contamination, respectively. The peak at 2050 cm⁻¹ remains unmatched. Doublet peaks around 2880 cm⁻¹ in S2: Ga2(TiO3)3-HT, matched C−H furnace contamination. Finally, all spectra except S4: Cp-Ti-HT exhibited a peak around 1610–1630 cm⁻¹, consistent with O−H bonds.

3.1.7. XPS

XPS analysis of S1: Na2TiO3, S2: Ga2(TiO3)3, and S3: Ga2(TiO3)3-HT samples was conducted (Fig. 4). The initial O 1s peak (Fig. 4A) at

<table>
<thead>
<tr>
<th>Sample</th>
<th>Database file</th>
<th>Calculated d spacing / Å</th>
<th>Database d spacing / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1: Na2TiO3</td>
<td>Sodium titanate (Na0.23TiO2) (ICDD PDF 00-022-1404)</td>
<td>3.70</td>
<td>3.65</td>
</tr>
<tr>
<td></td>
<td>Titanium (Ti) (ICDD PDF 00-044-1294)</td>
<td>2.28</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>Sodium titanate (Na4TiO4) (ICDD PDF 00-042-0513)</td>
<td>3.22</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.28</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.87</td>
<td>1.87</td>
</tr>
<tr>
<td>S2: Ga2(TiO3)3</td>
<td>Calcium titanate (CaTi2O5) (ICDD PDF 00-025-1450)</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>Sodium titanate (Na2TiO3) (ICDD PDF 00-037-0346)</td>
<td>3.27</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.83</td>
<td>1.87</td>
</tr>
<tr>
<td>S3: Ga2(TiO3)3-HT</td>
<td>Gallium titanate (Ga2TiO5) (ICDD PDF 01-070-1993)</td>
<td>3.50</td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>Calcium titanate (CaTi2O5) (ICDD PDF 00-025-1450)</td>
<td>2.88</td>
<td>2.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.82</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.32</td>
<td>2.35</td>
</tr>
<tr>
<td>S4: Cp-Ti-HT</td>
<td>Rutile (TiO2) (ICDD PDF 00-021-1276)</td>
<td>2.45</td>
<td>2.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.28</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.19</td>
<td>2.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.05</td>
<td>2.05</td>
</tr>
</tbody>
</table>
Deconvolution of O 1s for S1: Na₂TiO₃ demonstrated three peaks respectively. Each peak matched O 530.2, 531.6, and 532.9 eV, with area ratios of 75.0, 15.3, and 9.7%, respectively. This reduced to two peaks at 530.3 (49.3%) and 531.9 (50.7%) eV in S2: Ga₂(TiO₃)₃. However, a further shift to 530.7 eV in S2: Ga₂(TiO₃)₃-HT resulted in a surface Ga:P ratio close to 1:3, whereas S2: Ga₂(TiO₃)₃ reached 1.71 by 168 h. Furthermore, rod-like deposits were also seen on both samples at 24 and 72 h. Their composition, as delineated by EDX, consisted mainly of Ga and O, suggesting Ga₂O₃ had deposited. By 168 h, the surface morphology (Fig. 5E & F) showed an absence of both spherical and rod-like surface growths in S2: Ga₂(TiO₃)₃, and larger clusters of rod-like deposits had formed on S3: Ga₂(TiO₃)₃-HT.

A perceptible shift was noted in the Ti 2p doublet peak (Fig. 4D) for S3: Ga₂(TiO₃)₃-HT sample, was the only sample that exhibited a significant difference (p < 0.0001) from the TCP control. The dotted line in Fig. 7 shows this threshold at 70% signal intensity. The untreated S0: Cp-Ti sample demonstrated an average signal of 94.2%, with S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT showing average signals of 24.2% and 81.4%, respectively. Therefore, both S0: Cp-Ti and S3: Ga₂(TiO₃)₃-HT samples are above the viability threshold, with a clear reduction in cell viability for the S2: Ga₂(TiO₃)₃ sample.

3.1.8. Degradation and ion leaching

Fig. 5(A–F) demonstrated the surface alteration of S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT samples after degradation in 1 mL DMEM over 168 h. It is clear, compared to surfaces illustrated in Fig. 1, that surface deposition/growth occurred during degradation, as well as opening of the porous surface network. Spherical deposits were seen on both S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT at 24 and 72 h. EDX analysis of the deposits demonstrated their composition to be rich in Ca and P. Ca:P ratios were then taken, as demonstrated in Fig. 5G, with S3: Ga₂(TiO₃)₃-HT resulting in a surface Ca:P ratio close to 1:3, whereas S2: Ga₂(TiO₃)₃ reached 1.71 by 168 h. Furthermore, rod-like deposits were also seen on both samples at 24 and 72 h. Their composition, as delineated by EDX, consisted mainly of Ga and O, suggesting Ga₂O₃ had deposited. By 168 h, the surface morphology (Fig. 5E & F) showed an absence of both spherical and rod-like surface growths in S2: Ga₂(TiO₃)₃, and larger clusters of rod-like deposits had formed on S3: Ga₂(TiO₃)₃-HT.

A combination of EDX and ICP (Fig. 6) was used to identify the alteration of both surface and solution ion concentrations during DMEM degradation. Over 168 h, aqueous Ga ion concentrations gradually increased for S3: Ga₂(TiO₃)₃-HT (Fig. 6D) as expected, however, at a slower rate than S2: Ga₂(TiO₃)₃ (Fig. 6B), with a peak Ga ion concentration of 2.76 and 0.68 ppm for S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT, respectively. The error at 168 h in S2: Ga₂(TiO₃)₃ means quantification here was difficult. Additionally, S2: Ga₂(TiO₃)₃-HT, surface Ga concentration (Fig. 6A) decreased over the course of 168 h, whereas the S3: Ga₂(TiO₃)₃-HT sample (Fig. 6C) demonstrated a re-deposition of Ga during the later time points. For both S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT samples, aqueous Ga ion concentrations decreased between 0 and 168 h (Fig. 6B & D). Both surface Ca and P ion concentrations increased for S2: Ga₂(TiO₃)₃, however, S2: Ga₂(TiO₃)₃ (Fig. 6A) exhibited deposition and subsequent re-release during the 168 h period (Fig. 6C).

3.1.9. Cell studies

From ISO 10993-5:2009, the definition of a cytotoxic effect demonstrated by an NRU assay is a ≥30% reduction in cell viability from the non-treated cells (Tissue Culture Plastic (TCP) control). The dotted line in Fig. 7 demonstrates this threshold at 70% signal intensity. The untreated S0: Cp-Ti sample demonstrated an average signal of 94.2%, with S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT showing average signals of 24.2% and 81.4%, respectively. Therefore, both S0: Cp-Ti and S3: Ga₂(TiO₃)₃-HT samples are above the viability threshold, with a clear reduction in cell viability for the S2: Ga₂(TiO₃)₃ sample. It was shown through a One-way ANOVA, followed by the Bonferroni post-test that the S2: Ga₂(TiO₃)₃ sample, was the only sample that exhibited a significant difference (p < 0.0001) from the TCP control. Biofilm development assay results are shown in Fig. 8, with no significant difference being noted between the live or dead biomass on any of the samples. The presence of dead bacteria on the Ti control sample is expected due to the length of the incubation period. An antimicrobial effect would be shown either by a significantly reduced total signal (both live and dead) from either titanate structures compared to the S0: Cp-Ti control, or by a significant decrease in live (green) signal and subsequent increase in dead (red) signal. Neither of these effects was prevalent in the data shown and was also not observed when the experiment was repeated.
4. Discussion

4.1. Composition and topographical analysis by SEM, FEG-SEM, EDX, FTIR, XRD, XPS, and Raman

Ion-exchange reactions were a key development in the production of tailored, application specific titanate surfaces. This is due to the initial, layered sodium hydrogen titanate, formed from the NaOH treatment, allowing ion incorporation and substitution with Na\(^+\) ions already present. Not only are these surfaces able to release ions into the surrounding media, but they can also facilitate further ion-exchange reactions in vivo, allowing generation of amorphous calcium phosphate layers, or release of therapeutic or antimicrobial ions.

The nanoporous surface morphology exhibited by S1: Na\(_2\)TiO\(_3\) and S2: Ga\(_2\)(TiO\(_3\))\(_3\) was consistent with the only other gallium titanate study published [48] and the higher resolution presented here clearly shows interesting differences from the S0: Cp-Ti control, where no significant features were present. Not only are these surfaces able to release ions into the surrounding media, but they can also facilitate further ion-exchange reactions in vivo, allowing generation of amorphous calcium phosphate layers, or release of therapeutic or antimicrobial ions.

The XRD results suggested the initial hydrothermally produced (S1: Na\(_2\)TiO\(_3\)), and ion-exchanged layers (S2: Ga\(_2\)(TiO\(_3\))\(_3\)) were amorphous in nature, since no additional crystalline peaks, further to the S0: Cp-Ti control, were present, correlating with the diffuse ring patterns noted in RHEED (Fig. 2). This was to be expected as no heat-treatment had been conducted, therefore, the surface layer produced should be amorphous; crystallisation temperature \(\approx 500 \, ^\circ\text{C} [57]\). Smaller, less intense, peaks were noted in XRD, with the lower intensities potentially attributed to lower quantities of surface crystals, due to the temperature being below the stated crystallisation temperature of gallium titanate (\(\approx 1100 \, ^\circ\text{C} [58]\)). However, this evidence alone was not conclusive, due to significant overlap with rutile, to identify the formation of...
titanate layers, and hence RHEED analysis was also conducted. This enabled shallower beam penetration, of the order of a few tens of nanometers, as well as higher probing resolution (0.01–0.001 nm) [55].

Upon heat-treatment (S3: Ga2(TiO3)3-HT), the sample yielded new Bragg peaks corresponding to rutile: a characteristic phase transformation of titanium at >600 °C in oxygen, as anticipated [59]. Formation of rutile was also seen in the S4: Cp-Ti-HT sample, in the RHEED d spacing analysis, as well as two characteristic peaks detailed in FTIR (Fig. 3B), and three in Raman spectroscopy (Fig. 3A). Furthermore, smaller Bragg peaks at 26, 36, 38 and 55° 2θ from the XRD patterns, were deconvoluted as gallium titanate derivatives, partially confirming its formation. To avoid characterising just the rutile produced in S3: Ga2(TiO3)3-HT, as well as the Ti substrate in S1: Na2TiO3 and S2: Ga2(TiO3)3, and allow characterisation of solely the produced surface layers, RHEED was employed. RHEED has a similar probing depth to the XPS used and, therefore, provides an ideal technique to compare and

![M.D. Wadge et al. / Materials and Design 155 (2018) 264–277](image-url)

Fig. 5. (A, C, and E) FEG-SEM images of the surface of degraded S2: Ga2(TiO3)3 samples in 1 mL DMEM (diluted with 1:10 ratio of ultrapure water) at time points 24, 72, and 168 h, respectively. (B, D, and F) FEG-SEM images of the surface of degraded S3: Ga2(TiO3)3-HT samples at 24, 72, and 168 h, respectively. (G) Graph showing the alteration in Ca:P ratio on the surface of S2: Ga2(TiO3)3 and S3: Ga2(TiO3)3-HT during the degradation study. Ca:P rich nodules and Ga2O3 precipitates were observed.
corroborate results. As seen in Fig. 2D, RHEED demonstrates a clear diffraction pattern for rutile on S4: Cp-Ti-HT, and matches d spacing values from the database, as well as confirming the results from XPS (Fig. 4).

Rutile diffraction rings were not observed in S3: Ga₂(TiO₃)₃-HT. However, even with RHEED, it was noted that the S1: Na₂TiO₃, S2: Ga₂(TiO₃)₃, and S3: Ga₂(TiO₃)₃-HT samples exhibited a more diffuse pattern than S4: Cp-Ti-HT only, causing overlap and complicated the quantification. This diffuseness could be attributed to the amorphous sodium or gallium hydrogen titanate layers present. Despite the diffuse rings, quantification of d spacing values was possible for S1: Na₂TiO₃, S2: Ga₂(TiO₃)₃, and S3: Ga₂(TiO₃)₃-HT, which matched sodium titanate derivatives (Na₀.2₃TiO₂ and Na₄TiO₄); and calcium and sodium titanate variants (CaTi₂O₅ and Na₂TiO₃); and gallium and calcium titanate derivatives (Ga₂TiO₅ and CaTi₂O₅) also suggested by [48], respectively.

The evidence demonstrated through XRD and RHEED was supported by IR absorption spectroscopy (Fig. 3B), which demonstrated characteristic TiO₆ octahedron vibrations, Ti—O bond stretching and Ti—OH non-bridging bonds of titanate structures. Edge-sharing TiO₆ octahedra and Ti—O—Ti stretching were also present in the Raman analysis [60–62]. Additionally, XPS also supported titanate formation, through the presence of Ti⁴⁺—O bonding [63], which were ubiquitous across all samples, in both the Ti 2p and O 1s deconvolution, and are characteristic of titanate structures, as discussed by Takadama et al. [64]. Specifically for S1: Na₂TiO₃, there were no other FTIR absorption bonds corresponding to sodium titanate formation, however, this may be attributed to limitations on the FTIR spectrometer used, which made analysis lower than 600 cm⁻¹ difficult [65]. Nevertheless, FTIR ruled out formation of re-precipitated NaOH, due to the lack of characteristic O—H tension peaks around 3600 cm⁻¹ [66]. Despite this, Raman (Fig. 3A) and XPS analysis confirmed the presence of Na—O bonds, which are readily seen in sodium titanate structures [67]. The additional presence of O—H bending modes in Raman (as described by Oleksak et al. [68]), and —OH bonds in XPS, before and after heat-treatment, suggest amorphous sodium and gallium hydrogen titanate may also be present on the surface.

The shoulder exhibited between 800 and 900 cm⁻¹, shown in FTIR for S2: Ga₂(TiO₃)₃, may have corresponded to GaO(OH) vibrations and Ga—OH bending modes, which could be attributed to gallium hydrogen titanate formation prior to heat-treatment, as well as the Ga(OH) flakes noted in Fig. 1G [56,69]. Furthermore, peaks demonstrated by Raman spectrometry may correspond to gallium oxide, as shown by Zhao et al. [70], Rao et al. [71], and Gao et al. [72], or derivatives of gallium titanate. The Raman peak at 700 cm⁻¹ remains as a shoulder in S3: Ga₂(TiO₃)₃-HT, and correlates with the GaO(OH) flakes seen in Fig. 1G. Gallium titanate formation is also confirmed by XPS analysis, with the Ga 2p 3/2 peak position at ≈1118.5 eV relating to Ga—O in its Ga⁴⁺ state, which are doped at various characteristic Ti⁴⁺ sites, as detailed by Deng et al. [73]. Furthermore, the presence of Ti—O Raman bonds in S₂: Ga₂(TiO₃)₃, suggest gallium titanate formation [74]. A significant alteration, which correlates well with the EDX results previously mentioned, is the reduction in the Na 1s peak in XPS for both S₂: Ga₂...
(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT, demonstrating complete Na replacement, and the subsequent formation of gallium titanate.

In addition to titanate formation, broad absorption peaks from 3000 to 3500 cm⁻¹, seen in both S1: Na₂TiO₃ and S2: Ga₂(TiO₃)₃, can be ascribed to H—O—H stretch bonds of any remaining surface, or chemisorbed/interlamellar water, since this stage was prior to the heat-treatment step [75]. The removal of these peaks in both heat-treated samples: S3: Ga₂(TiO₃)₃-HT and S4: Cp-Ti-HT, support this postulation and is further backed up by Shiropur et al., who showed peak elimination during dehydration [60]. Interestingly, FTIR demonstrated a peak at 1100 cm⁻¹ in both S1: Na₂TiO₃ and S2: Ga₂(TiO₃)₃, potentially matching Ti—O—C vibrations, which is unexpected, as the carbon location would be in place of either gallium or sodium in the titanate structure [76]. It is evident from the heat-treatment stage, through the generation of doublet peaks at 2800 cm⁻¹ (S3: Ga₂(TiO₃)₃-HT) and the shoulder at 2350 cm⁻¹ in FTIR, matching C—H bonds and atmospheric CO₂, respectively, that carbon contamination on the surface of the samples is present and unavoidable [77].

4.2. Surface degradation and ion release

During submersion in DMEM, opening of the porous network in the titanate surfaces was observed. Furthermore, spherical and rod-like deposits, which through EDX analysis were found to be formed of Ca:P and Ga:O, respectively, were also noted (Fig. 5). Morphologically, the rod-like Ga:O deposits look similar to those generated by Zhao et al. and Shah et al. [78,79]. Deposition may have occurred due to oversaturation of the surrounding solution, however, further studies would be needed to confirm this postulation. Additional EDX analysis was conducted on the Ca and P deposits to understand the Ca:P ratio, and whether these deposits were similar to HA. For S2: Ga₂(TiO₃)₃, the Ca:P ratio increased significantly above 1.8 within 6 h and gradually plateaued at 1.71 by 7 days. This is in stark contrast to the heat-treated sample (S3: Ga₂(TiO₃)₃-HT), which had a Ca:P ratio of ~1.42 at 6 h and reached a final ratio of 1.34 by 7 days. Stoichiometric HA contains a Ca:P = 1.67, with calcium deficient and calcium rich HA having ratios of <1.67 and >1.67, respectively [80]. Correlating this with the Ca:P generated on both samples, S2: Ga₂(TiO₃)₃ and S3: Ga₂(TiO₃)₃-HT are calcium rich and calcium deficient, respectively. Studies conducted by Kizuki et al. demonstrated the relative propensity for ion inclusion into the titanate layer for Ca²⁺ and Na⁺ [81]. The studies concluded that, even with a calcium contamination of 0.0005% in the sodium containing solution, divalent Ca²⁺ ions would preferentially enter into the structure, as it has a more potent electrostatic attraction to negative TiO₆ [82]. The authors hypothesise that the calcium contained within the solution, preferentially ion-exchanged into the surface layer due to its relatively higher propensity, as demonstrated through literature studies investigating Ca²⁺ ions preferentially exchanging into the titanate structure [27,28,83]. As S2: Ga₂(TiO₃)₃ has a less stable layer compared to S3: Ga₂(TiO₃)₃-HT, due to the increased release rate of Ga ions, this explains why there is a higher Ca content on S2: Ga₂(TiO₃)₃.

The opening of the porous network, as well as the deposition of Ca:P and Ga₂O₃ exhibited in the micrograph images (Fig. 5) correlates with the ICP and EDX analysed ionic alterations on the sample’s surface and in solution. As shown in Fig. 6, S3: Ga₂(TiO₃)₃-HT released gallium at a much slower rate than S2: Ga₂(TiO₃)₃, suggesting the heat-treatment had a significant effect on the stability of the titanate surface generated. Moreover, the peak Ga solution concentration was much greater for S2:

![Image](image_url)

**Fig. 8.** (A, B, and C) LIVE/DEAD staining maps for S0: Cp-Ti, S2: Ga₂(TiO₃)₃, and S3: Ga₂(TiO₃)₃-HT, respectively. Live bacteria are stained green, with dead bacteria stained red, as indicated. (D) Live and dead biomass from a 3 day culture of *S. aureus* analysed via COMSTAT. There is no significant difference between the live or dead values between the samples (2 way ANOVA). The experiment was repeated and the same trends observed (*n* = 3; error bars in S.E.M.).
Ga$_2$(TiO$_3$)$_3$ (2.76 ppm; day 3) compared to S3: Ga$_2$(TiO$_3$)$_3$-HT (0.68 ppm; day 7). Additionally, the trend in surface concentration of Ga in Fig. 6 agrees well with the micrographs presented in Fig. 5. The S2: Ga$_2$(TiO$_3$)$_3$ sample exhibited an overall decrease in Ga ions with no deposition occurring, whereas S3: Ga$_2$(TiO$_3$)$_3$-HT demonstrated a deposition of Ga back onto the surface after 24 h, with a large proportion of Ga:O deposits. Furthermore, the decrease in solution ionic concentrations of Ca and P, as well as the overall increase of these ions on S2: Ga$_2$(TiO$_3$)$_3$, relates to the deposition of Ca:P deposits seen in Fig. 5. The anomalous re-release of Ca and P from the surface of S3: Ga$_2$(TiO$_3$)$_3$-HT, which does not match the solution concentration, could be due to detachment of Ca:P precipitates, which are not detectable via ICP. Distinction between Ca ions penetrating into the titania layer and deposition on the surface was not possible with the techniques used, hence further studies would be needed.

The mechanism for amorphous calcium phosphate formation, and subsequent apatite maturation, has been explained previously [27,84]. The surface titania layers, containing positive metallic ions, with this case being Ga$^{3+}$, facilitate ion exchange between H$_2$O$^+$ (hydronium) ions and Ga$^{3+}$. This exchange generates Ti—OH bonds upon the top surface of the titania layers, generating an overall negative surface charge. This negative charge allows Ca$^{2+}$ ions to preferentially ion-exchange into the surface. High concentration of Ca$^{2+}$ ions on the surface generates an overall positive surface charge, allowing phosphate ions present within the DMEM solution to be attracted to the surface, generating calcium phosphate precipitates (Fig. 5). Since S3: Ga$_2$(TiO$_3$)$_3$-HT contained a heat-treatment stage and, therefore, had a more stable surface layer, Ga release was much lower than S2: Ga$_2$(TiO$_3$)$_3$ (Fig. 6), which evidently resulted in lower consumption of Ga ions from the DMEM onto the surface (Figs. 5 & 6). This is evident in the calcium-deficient Ca:P precipitates present on S3: Ga$_2$(TiO$_3$)$_3$-HT, as well as the smaller quantity of precipitates present on the surface (Fig. 5).

Although the relationship between heat-treatment temperatures and Ga release was not investigated here, the conversion of a sodium titianate hydrogel following heat-treatment was the subject of a previous study by Kim et al. Their findings showed that the progressive increase in heat-treatment temperatures converted the gel into an amorphous and crystalline sodium titania at 400 and 700 °C, respectively, reducing its reactivity and propensity to form apatite in simulated body fluid [83]. It is postulated that Ga ion release would decline with increases in heat-treatment temperatures in a similar manner.

4.3. Cytotoxicity and antimicrobial assessment

Initial evaluation on the effect of titania surfaces on human (MG-63) cells has been performed via a Neutral Red Uptake assay. Upon exposure to media, which had been in contact with the samples for 7 days, significant reduction in cell viability was only shown for S2: Ga$_2$(TiO$_3$)$_3$, with the performance of S0: Cp-Ti, S3: Ga$_2$(TiO$_3$)$_3$-HT and cells exposed to untreated media showing no significant differences (Fig. 7). From the ICP analysis, the maximum Ga release for the S2: Ga$_2$(TiO$_3$)$_3$ and S3: Ga$_2$(TiO$_3$)$_3$-HT samples were 2.76 and 0.68 ppm (39.6 and 18.6 μM), respectively. Although these concentrations are lower than those commonly seen in the literature for Ga toxicity to human cells, the hypothesis that the heat-treatment stabilising the rate of gallium release is supported by these results [85,86]. The toxicity of Ga can also be effected by local Fe concentrations and any binding molecules, which can promote Ga uptake into the cells. It is also possible that a toxic pH was caused by the eluant of the S2: Ga$_2$(TiO$_3$)$_3$ samples during ion-exchange within the structure; an effect which is lost after heat-treatment.

In this pilot study, S. aureus was used as it is a clinically relevant pathogen commonly associated with nosocomial, and orthopaedic biofilm infections, occurring in as many as 75% of joint infections [87–89]. Although Ga has been demonstrated to be antimicrobial against a wide variety of pathogens, its efficacy varies over a wide range of inhibitory concentrations (μM–mM) specific to each bacterial strain. An antimicrobial effect of gallium titinate structures against A. baumannii has been recently demonstrated by Yamaguchi et al. [48]. A. baumannii has been found to be particularly susceptible to Ga (2–100 μM), whereas S. aureus is relatively more resistant compared to other species (0.32–5.12 mM) [44,90]. Although the concentration of gallium used to produce these structures was far higher than in the Yamaguchi study, these results suggest that it has still fallen short of the minimum inhibitory concentration to prevent a S. aureus infection. In DMEM, the Ga release after 6 h was 1.04 and 0.32 ppm for S2: Ga$_2$(TiO$_3$)$_3$, and S3: Ga$_2$(TiO$_3$)$_3$-HT, respectively (15 and 4.6 μM in 1 mL solution), which falls well below the toxic concentrations for S. aureus, in addition to being considerably lower than concentrations clinically used [91]. However, upon reflection, the authors feel it is necessary to conduct a further, more comprehensive, study to fully elucidate the antimicrobial status of gallium titinate surfaces against S. aureus and other common nosocomial pathogens.

5. Conclusions

Formation of gallium titinate surfaces through sequential hydrothermal NaOH, Ga(NO$_3$)$_3$ and subsequent heat-treatments, was successful. Full characterisation of the produced gallium titinate surfaces was conducted, using FEG-SEM, RHEED, XPS, FTIR, EDX, Raman, and ICP methodologies. Significant morphological changes were demonstrated at high-resolution on titanium surfaces upon hydrothermal treatment in NaOH, ion-exchange in Ga(NO$_3$)$_3$, and subsequent heat-treatment. Furthermore, the antimicrobial and cytotoxic nature of the produced surfaces were assessed via Neutral red and LIVE/DEAD analyses. In addition to the Ga ion’s ability to substitute into the sodium titinate structure, the surface layer enables release of Ga ions into the surrounding environment. However, further testing against a wider range of relevant pathogens is required in order to demonstrate the concentrations of Ga necessary for these surfaces to be clinically effective. It is also clear that the heat-treatment conducted on the gallium titinate surface resulted in a more stable layer that released Ga ions at a slower rate: 2.76 compared to 0.68 ppm for S2: Ga$_2$(TiO$_3$)$_3$ and S3: Ga$_2$(TiO$_3$)$_3$-HT, respectively. Further to this, the incorporation of Ca/P ions on the surface was much lower on the heat-treated surface (S3: Ga$_2$(TiO$_3$)$_3$-HT), generating a calcium deficient amorphous precipitate (Ga:Ca = 1.34), relative to crystalline HA, and as compared to the calcium rich (Ga:Ca = 1.71) precipitate deposited on the surface of S2: Ga$_2$(TiO$_3$)$_3$.

If additional assessments can indicate microbiological and further osteogenic efficacy, such surfaces may be suitable candidates as an orthopaedic alternative. The production design, which utilised low temperature Ga ion-exchange reactions, will enable tailorable and cost effective antimicrobial surfaces that can potentially be used to coat both surfaces and internal porosities of orthopaedic prosthetics at commercial scales; a key design improvement.

Acknowledgements

This work was supported by the Engineering and Physical Sciences Research Council [grant numbers EP/K029592/1, EP/L022494/1] and the EPSRC Centre for Innovative Manufacturing in Medical Devices (MeDe Innovation). The authors would like to gratefully acknowledge the Nanoscale and Microscale Research Centre (nmRC) at the University of Nottingham for SEM, FEG-SEM and Raman access, as well as Dr. N. C. Neate for his help in RHEED acquisition and analysis, and Dr. G. A. Rance for Raman technical assistance. We would also like to gratefully acknowledge Saul Vazquez Reina for assistance with ICP-MS analysis.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.


