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Functionalisation of Ti6Al4V components fabricated using selective laser melting with a bioactive compound



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ABSTRACT

Surface modification of an implant with a biomolecule is used to improve its biocompatibility and to reduce post-implant complications. In this study, a novel approach has been used to functionalise phosphonic acid monolayers with a drug. Ti6Al4V components fabricated using selective laser melting (SLM) were functionalised with Paracetamol (a pharmaceutically relevant biomolecule) using phosphonic acid based self-assembled monolayers (SAMs). The attachment, stability of the monolayers on the SLM fabricated surface and functionalisation of SAMs with Paracetamol were studied using X-ray photoelectron spectroscopy (XPS) and surface wettability measurements. The obtained results confirmed that SAMs were stable on the Ti6Al4V surface for over four weeks and then began to desorb from the surface. The reaction used to functionalise the phosphonic acid monolayers with Paracetamol was noted to be successful. Thus, the proposed method has the potential to immobilise drugs/proteins to SAM coated surfaces and improve their biocompatibility and reduce post-implant complications.

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1. Introduction

Selective laser melting (SLM) is a metal-based additive manufacturing (AM) technique, which is gaining considerable interest for biomedical applications due to its ability to fabricate parts with customised and complex geometries directly from 3D model data [1–5]. However, limitations are present including the materials currently available and the surface quality of fabricated parts. Some of the biomaterials that can be processed by SLM include titanium and its alloys (such as Ti6Al4V and Ti6Al7Nb), cobalt–chromium alloy and 316L stainless steel. Control over the surface chemistry of an implant is essential since it governs the surface chemical reactions. It is not always possible to achieve the desired surface chemistry directly from the manufactured part and hence surface modifications are performed to obtain the desired surface chemistry.

In recent years, surface modification has been performed not just to improve the surface finish of the implant but also to make the implants biocompatible and a drug carrier [6–12]. For example, to reduce the restenosis rate in drug eluting stents (DES), the stents are coated with polymers containing drugs such as Paclitaxel. In addition, to improve

biocompatibility, the implants are coated with biocompatible proteins, ceramics and other biomaterials. Hence by changing the physicochemical characteristics, material and biological responses are altered [13].

Most implants are coated or surface modified with biocompatible materials using techniques such as dip-, spray- or spin-coating or solvent casting [14,15]. Implants coated with these conventional coating techniques have drawbacks including surface heterogeneity in the type and distribution of functional groups, hydrophilic or hydrophobic domains and surface roughness [16]. Limitations of using polymeric carriers for biomedical applications include fissures, cracks and waviness of the coating (leading to uneven drug distribution), inflammatory and hypersensitivity reactions [17]. Thus a coating technique that offers unprecedented control over the location and orientation of chemical groups/biomolecules on the surface is essential to cater for current needs.

Self-assembled monolayers (SAMs) are amphiphilic molecules with head and tail groups. Typically the head group is attached to the surface that needs modification and the tail group is used to introduce functionality [18]. SAMs are formed on a substrate by the adsorption of molecules from a solution and are usually nanosized adding only a few nanometres of thickness (depending on the alkyl chain) to the implant surface [19]. SAMs can be designed at the molecular level to be biologically inert and can be expanded uniformly (to cover the whole implant surface) with the implant surface maintaining their assembly, structural integrity and chemical composition. SAMs are inexpensive and versatile

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for applications including control of wetting and adhesion, chemical resistance, nano-fabrications, molecular recognition for sensors, drug delivery and biocompatibility [20,21].

Various biomolecules including proteins, peptides, carbohydrates, antibodies and therapeutics have been attached to SAMs for biomedical applications [22]. Since SAMs can serve as a localised drug delivery system, the use of SAMs to attach drugs to biomedical implants is gaining considerable interest. There are several literature examples of the functionalisation of monolayers with drugs [11,17,23,24]. However, these drugs are either physisorbed or both physisorbed and covalently bound.

A method was proposed by Mani et al. [17] to successfully bind Paclitaxel to SAM coated surfaces; however, it has a number of limitations. In their procedure, a microdrop method was used to drop the drug solution on to the cobalt–chromium surface. It was reported that from the 25 µg of drug placed on the surface (1 cm²), only 25% of the drug was adsorbed to the surface and the rest was washed away by solvent cleaning. Although a certain amount of the drug can be recovered by recycling, this method can be time consuming and expensive. Also it should be noted that scaling up of this method will be difficult since coating drugs on implants with complex geometries using microdrop deposition would be challenging. Furthermore, the drug adsorbed on the surface was observed to be crystallised. Crystallisation of drugs on an implant surface can potentially increase the surface roughness of the implant and may cause post-implant complications. Also due to crystallisation, controlling drug release from the implant surface will be problematic.

In this study, one-to-one binding of a drug directly to monolayers adsorbed on a SLM surface was studied. The surface of a SLM-manufactured part was used due to the increased interest in the use of SLM to fabricate biomedical implants [3,25–27]. By allowing the monolayers to react with drugs under optimal reaction conditions, drugs can be covalently bound to the monolayers. Such binding can avoid crystallisation of drugs on the implant surface and reduce the loss of drugs whilst coating. In this study, the stability of phosphonic acid monolayers on a Ti6Al4V surface was investigated. Following the stability of the monolayers, a novel approach was used to bind Paracetamol covalently to monolayers adsorbed on the SLM fabricated Ti6Al4V surface.

2. Materials and methods

2.1. Materials

Gas atomised grade 5 titanium alloy, Ti6Al4V powder, with an average particle size distribution (volume weighted) of 33.35 µm was purchased from LPW Technology Ltd., UK. Silicon carbide grits, polishing cloth, diamond paste and extender solutions were supplied by Buehler (Buehler-met® II). 16-Phosphano-hexadecanoic acid (16-PhDA) (97%) and Paracetamol (analytical grade) used for the surface modification were purchased from Sigma-Aldrich, UK. Thionyl chloride (97%), tetrahydrofuran (THF), hydrogen peroxide (30%) and sulphuric acid (98%) used for the cleaning and surface modification steps were also purchased from Sigma-Aldrich, UK. THF used for binding Paracetamol to monolayers was dried using benzophenone/sodium under argon atmosphere. This is referred in this study as “dry THF”.

2.2. Methods

2.2.1. Design and fabrication

Cuboidal Ti6Al4V parts with dimensions 10 × 10 × 3 mm were fabricated in a SLM 250 laser melting machine (Renishaw, UK) using a laser power of 200 W, hatch spacing of 100 µm, exposure time of 200 µs and a layer thickness of 50 µm. A Meander scan strategy was used to build the part. The theory behind the SLM process and its working principles can be found in literature [25]. After fabrication, the parts were removed from the build plate and sonicated in deionised water.

2.2.2. Sample preparation

The SLM-built samples were mechanically polished using a series of silicon carbide grits including 220 µm, 400 µm, 600 µm, 800 µm and 1200 µm diameter, for 5–7 min. These surfaces were then polished for 3 min with a polishing cloth using 6 µm and 1 µm diamond pastes. The mechanically polished surfaces were rinsed with deionised water and then immersed in a mixture of sulphuric acid, hydrogen peroxide and water in the ratio of 1:1:5 respectively for 15 min to remove surface contaminants. Finally the surfaces were sonicated in deionised water for 30 min (twice) to remove any traces of residual acids.

2.2.3. Self-assembly of monolayers

A solution immersion deposition method was used to coat 16-PhDA phosphonic acid SAMs on the mechanically polished Ti6Al4V plates. SAMs were deposited on the sample surfaces using a procedure previously reported in the literature [17]. Briefly, 1 mM solution of 16-PhDA in 150 ml THF was prepared. After 24 h, the samples were removed from the THF and any residual solution was allowed to evaporate in air. Without rinsing, the samples were immediately transferred to an oven maintained at 120 °C. After 24 h, the SAM-coated specimens were removed from the oven and allowed to cool to room temperature before ultrasonication in THF and deionised water for 1 min each.

2.2.4. Stability studies

The stability of the phosphonic acid monolayers assembled on the SLM fabricated Ti6Al4V surfaces was studied by immersing the SAM-coated samples in 20 ml of 10 mM Tris–HCl buffer solution (TBS) with a pH of 7.4. The samples were incubated at 36.5 °C for 6 weeks. The samples were removed from the TBS solution at 1, 2, 4 and 6 week time intervals and ultra-sonicated with deionised water for 1 min and dried with compressed air before characterisation.

2.2.5. Drug attachment

The SAM coated samples were transferred to a round bottomed flask (RBF) with the SAM coated surface facing upwards. The apparatus consisted of a RBF fitted to a reflux condenser. The entire reaction was carried out in a nitrogen atmosphere. The apparatus was dried using a flame/heat gun (an apparatus that produces flame) before use to remove any moisture. 20 ml of anhydrous THF was added to the RBF containing the samples through a side tube followed by the addition of 1 mM of thionyl chloride (SOCl₂). Care was taken to ensure that the mixture of THF and SOCl₂ added to the RBF covered the sample surface. The RBF together with the samples and SOCl₂ mixture were sonicated for an hour before syringing out the mixture from the RBF. Now the SOCl₂ reacted surfaces were sonicated thrice (for 2 min each time) using excess anhydrous THF without exposing the surfaces to the atmosphere. If exposed to the atmosphere, the chlorinated SAMs would have reacted with atmospheric air moisture and the acid chloride would have hydrolysed back to the carboxylic acid. After rinsing the samples, 5 mM of Paracetamol in 20 ml of anhydrous THF was added to the RBF and sonicated for an hour. Finally, the surfaces in the RBF were sonicated with an excess of anhydrous THF and deionised water thrice (each for 2 min) and characterised using XPS. Fig. 1 shows a schematic of the chemical reaction that took place at each step to bind the drug to the monolayers.

2.2.6. Surface characterisation

2.2.6.1. Surface morphology. The surface morphology of the SLM fabricated samples was imaged using a LEO 440 scanning electron microscope (SEM). SEM was operated at an extra-high tension (EHT) power of 10 kV and micrographs of the surfaces were obtained at magnifications including 50× and 500×.

2.2.6.2. Surface roughness. Surface roughness (Ra) measurements of the SLM as-fabricated samples and the mechanically polished

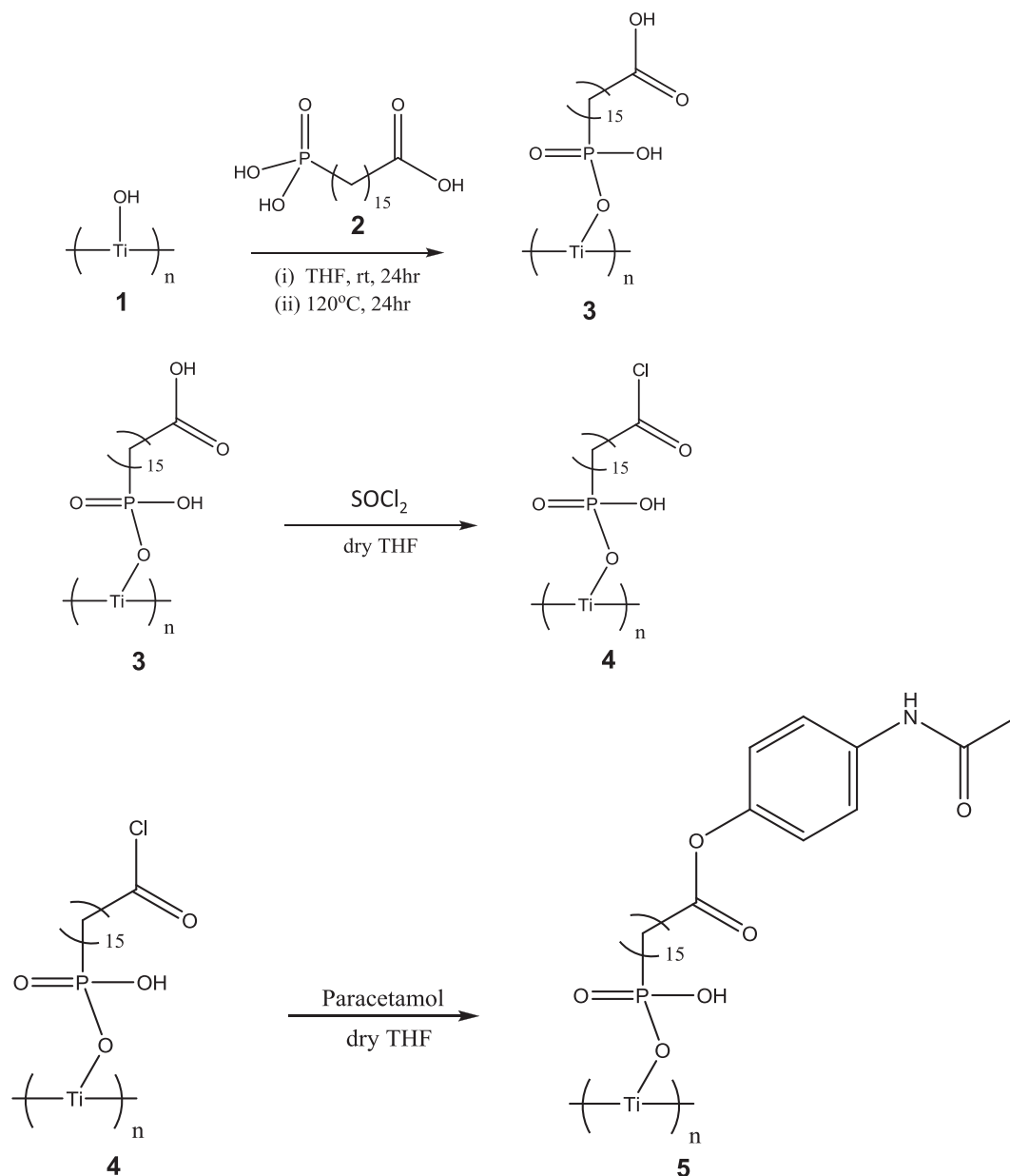


Fig. 1. General reaction scheme to functionalise 16-PhDA SAMs with Paracetamol. Label description: 1. Hydroxylated titanium surface; 2. 16-Phosphano-hexadecanoic acid; 3. SAMs adsorbed on a Ti6Al4V surface; 4. Chlorinated surface; 5. Ti6Al4V surface functionalised with Paracetamol.

samples were obtained using an Alicona InfiniteFocus® optical 3D measurement device. Surface roughness patterns were obtained from a $175 \mu\text{m} \times 175 \mu\text{m}$ square area of the sample. Surface roughness

was measured for five samples and averaged. The data acquired was processed using the in-built software within the Alicona InfiniteFocus®.

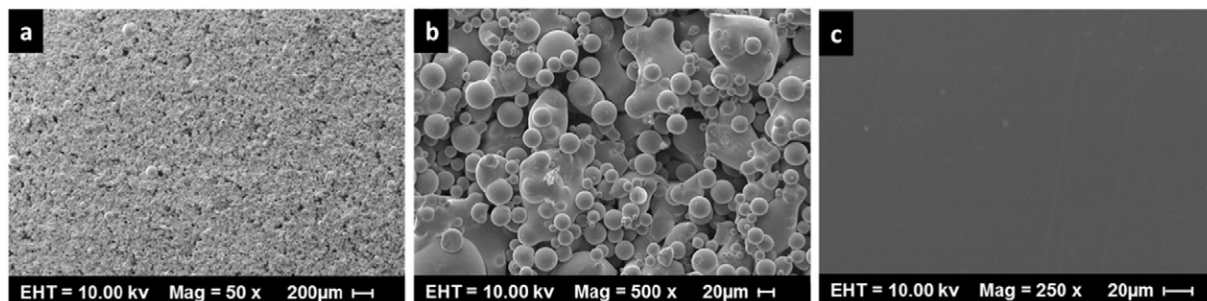


Fig. 2. SEM micrographs showing the surface morphology of a typical SLM as-fabricated (a and b) Ti6Al4V surface (Magnifications 50× and 500×) and a mechanically polished surface (c).

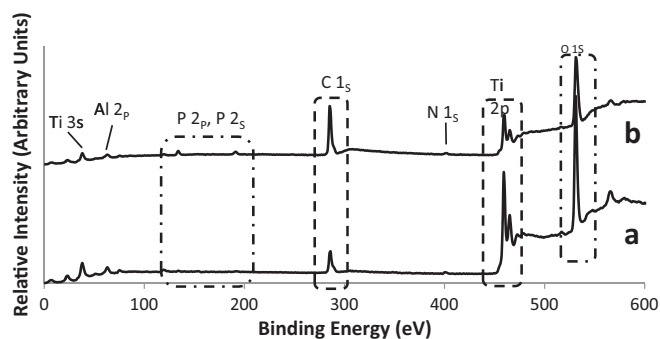


Fig. 3. Survey spectra showing the change in surface chemistry of a Ti6Al4V surface after SAM attachment. (a) Control surface without SAM coating and (b) after 16-PhDA SAM coating.

2.2.6.3. Surface wettability. Surface wettability of the samples before and after surface modification (SAM attachment and drug attachment) was determined by static water contact angle measurement using a contact angle goniometer (OCA-20). A drop volume of 2 μ l was placed on the surface and allowed to settle for approximately 5 s. The contact angle formed by the water drop on the sample surface was measured using the inbuilt software SCA 20. Contact angles were measured in five distinct spots within the sample surface and averaged.

2.2.6.4. Surface chemistry. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Thermo Scientific K-Alpha ESCA with a probing spot size of 400 μ m. Using aluminium (Al) K α monochromated radiation at 1486.6 eV, photoelectrons were collected using a take-off angle of 90°. In a constant analyser energy mode, survey spectra were collected at a pass energy of 100 eV and high resolution spectra were obtained at a pass energy of 20 eV. Peak deconvolution was performed using Gaussian–Lorentzian curves to investigate the different chemical states of the detected elements. A built-in Thermo Scientific Avantage data system was used for data acquisition and processing. Previously reported works and the National Institute of Standards and Technology (NIST) database were used to identify the spectral lines.

3. Results and discussion

3.1. Surface morphology

The surface morphology of a typical SLM-fabricated Ti6Al4V sample is shown in Fig. 2. Partially sintered particles observed in the micrograph show that the SLM fabricated sample was rough. The average surface roughness (Ra) obtained using a surface profilometer was 17.6 ± 3.7 μ m. The reason for such sintering of particles to the surface could be due to multiple factors including as a natural consequence of building parts from a powder bed, equipment based process parameters such as laser power, and laser beam spot size [28–30].

Some biomedical applications require a smooth surface (such as stents) to avoid cellular damage and some require a rougher, porous surface to promote cell adhesion and tissue integration and regeneration [13]. Although the SLM as-fabricated parts cannot be used for applications that require a smooth surface finish, they could be used where a

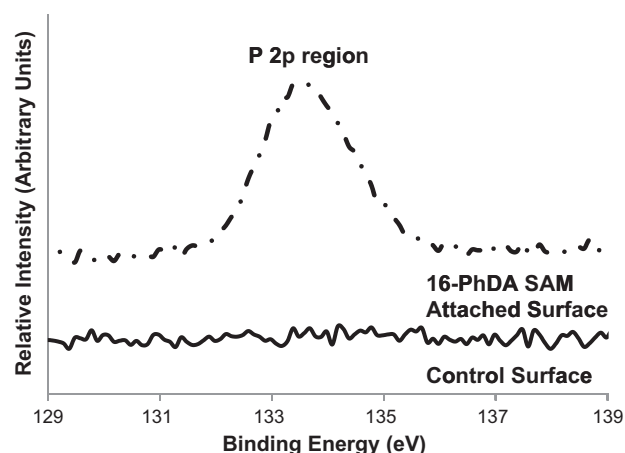


Fig. 4. High resolution spectra of phosphorous 2p region confirming the attachment of 16-PhDA monolayers to the Ti6Al4V surface.

porous surface is required. However, the SLM-produced surfaces are porous due to partially and loosely sintered particles. These particles could detach from the surface and can cause acute and chronic effects.

In addition, if particles detach after monolayer assembly, areas will be left without drug attachment since there will be no underlying monolayer to functionalise. XPS and contact angle measurements are sensitive to rough surface and hence, the use of SLM as-fabricated surface will affect the surface characterisation results. Hence in this study, the SLM fabricated surfaces were mechanically polished to remove the partially-sintered particles and to attain a smooth surface before surface modification and functionalisation with a drug. The average surface roughness (Ra) of the mechanically polished surfaces obtained using a surface profilometer was 0.437 ± 0.045 μ m.

3.2. Monolayer attachment

Fig. 3 shows the survey spectra obtained from the mechanically polished Ti6Al4V surface before and after surface modification using 16-PhDA SAMs. The spectra clearly indicate the introduction of a metal phosphonate peak at 133.3 eV showing that the surface has been modified with 16-PhDA monolayers. This metal phosphonate peak is due to the formation of Ti–O–P bonds after SAM attachment [31,32]. This has further been justified by an increase in the intensity of carbon and a reduction in the intensity of oxygen, titanium and aluminium after surface modification (due to the limited penetration depth of XPS measurements, less of the underlying metal and oxide is detected).

Table 1 shows the relative atomic composition obtained for the Ti6Al4V surface before and after surface modification. For a surface modified using 16-PhDA, the expected ratio of C:P is 16:1 and in the current study, it is observed to be nearly the same (i.e. 15.6:1). The small variation is likely due to errors in the integration of the small phosphorous peak. High resolution spectra obtained for the phosphorous 2p region showed the formation of metal–phosphonate bond at 133.6 eV (Fig. 4) confirming the modification of the surface with monolayers [31].

Table 1

Relative atomic percentage of the elements detected in XPS.

Sample	Relative atomic percentage								
	C	O	N	Ti	Al	V	P	Cl	S
Control	21.8 \pm 1.7	54.5 \pm 2.1	0.2 \pm 0.8	18.2 \pm 0.9	4.7 \pm 0.7	0.6 \pm 0.2	0	0	0
16-PhDA SAM	51.1 \pm 2.3	32.9 \pm 1.4	2.0 \pm 0.5	8.7 \pm 1.1	1.8 \pm 0.7	0.3 \pm 0.1	3.3 \pm 0.5	0	0

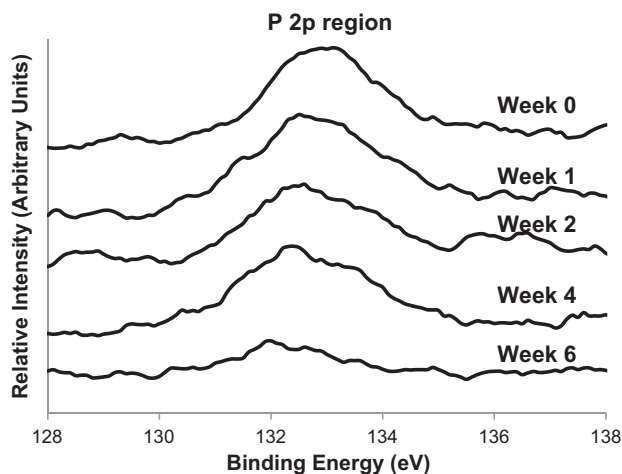


Fig. 5. XPS spectra for the phosphorous 2p region showing the stability of monolayers on the SLM fabricated Ti6Al4V surface at various time intervals.

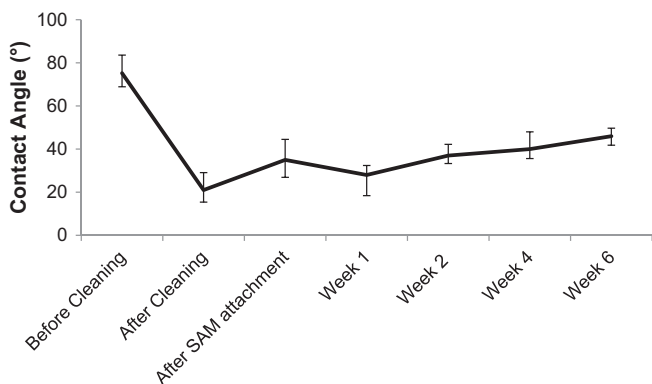


Fig. 6. Static water contact angles formed on the Ti6Al4V surface before and after SAM coating.

The average static contact angles obtained from the surface before and after surface modification with SAMs were $23.7^\circ \pm 3.4^\circ$ and $35.1^\circ \pm 7.1^\circ$ respectively. The contact angles can be observed to increase after SAM attachment showing a change in the surface wettability. However, since 16-PhDA SAMs are hydrophilic, displaying a carboxylic acid (COOH) terminal group, the surface remained highly wettable even after surface modification.

3.3. Stability studies

Stability of the 16-PhDA monolayers on the SLM fabricated Ti6Al4V surface was studied by immersing the SAM coated samples into Tris–HCl buffer solution. Fig. 5 shows the high resolution XPS spectra for the phosphorous 2p region of the samples soaked in the buffer solution for different time intervals. It can be witnessed from the spectra that the metal phosphonate peak at 133.3 ± 0.6 eV retained its position at the same binding energy for the whole duration of immersion in the buffer solution. However, its intensity and relative atomic composition decreased over the course of the experiment showing desorption of monolayers from the Ti6Al4V surface. Although the desorption of monolayers was noted to occur after four weeks, a small amount of monolayers was observed at the end of six weeks. The phosphorous 2p peak showed a small peak shift upon immersing the substrate in Tris–HCl buffer solution. This may be due to the charging effect of the substrate during XPS characterisation [33].

A gradual increase in the contact angle was noted after immersing the samples in Tris–HCl buffer solution (although this change has low significance compared to the standard deviations of individual measurements). This may be attributed to a small amount of desorption (Fig. 6) leading to the change in the assembly pattern of the monolayers. Thus, both the XPS characterisation and static water contact angle measurements confirm that 16-PhDA monolayers are relatively stable on the Ti6Al4V surface for more than the 28 day time period when soaked in Tris–HCl buffer solution. A previous study showed that 16-PhDA SAMs are stable on both SLM as-fabricated (SLM-AF) surface and a mechanically polished SLM surface (SLM-MP) for over 28 days [34]. This study also revealed that the surface roughness did not significantly affect the stability of the 16-PhDA monolayers formed on these rough (SLM-AF) and smooth (SLM-MP) surfaces.

3.4. Functionalisation of 16-PhDA monolayers with Paracetamol

After studying the stability of monolayers on the Ti6Al4V surface, a sample drug (Paracetamol) was used to functionalise the SAMs. The reactive carboxylic acid group at the tail end of 16-PhDA SAMs was reacted with thionyl chloride (SOCl_2) to form an intermediate acid chloride compound with $-\text{COCl}$ as the terminal group. This group was then allowed to react with the hydroxyl ($-\text{OH}$) group of Paracetamol to bind the drug covalently to the monolayers. The whole reaction step was performed in an inert atmosphere. Anhydrous THF was used to prevent the SOCl_2 reacting with moisture in the atmosphere to form HCl that could corrode the metal surface and also hydrolyse the $-\text{COCl}$ to inactive $-\text{COOH}$.

Fig. 7 shows the survey spectra for the Ti6Al4V sample before and after drug attachment. The corresponding atomic percentages are

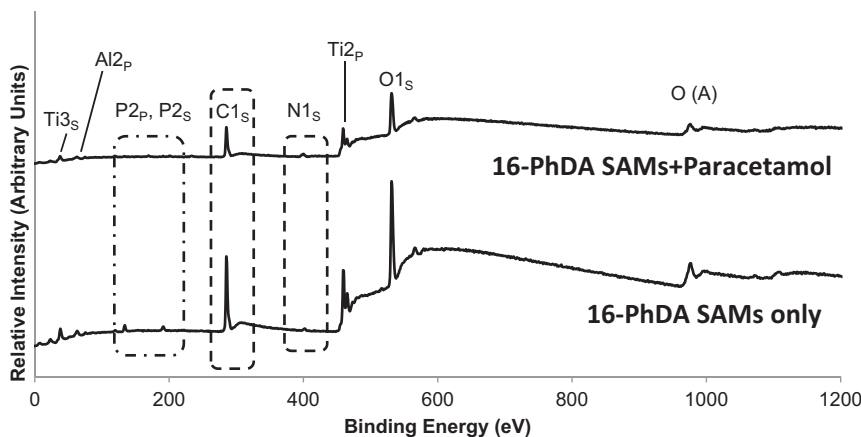


Fig. 7. Survey spectra obtained using XPS for the SAM coated and Paracetamol coated surfaces. Changes in the intensity of the detected elements before and after drug coating indicate a change in the surface chemistry.

Table 2

Relative atomic percentage of elements detected by the XPS on SAM coated and Paracetamol coated Ti6Al4V surface.

Sample	Relative atomic percentage								
	C	O	N	Ti	Al	V	P	Cl	S
16-PhDA SAM	51.1 ± 2.3	32.9 ± 1.4	2.0 ± 0.5	8.7 ± 1.1	1.8 ± 0.7	0.3 ± 0.1	3.3 ± 0.5	0	0
SAM + Paracetamol	48 ± 1.8	35.8 ± 1.6	4.5 ± 0.7	7.8 ± 1.3	1.1 ± 0.5	0	1.2 ± 0.4	0.8 ± 0.2	0.9 ± 0.1

given in Table 2. It can be clearly noted that there is a significant change in the surface chemistry of the SAM-coated Ti6Al4V surface after the attachment of Paracetamol. The ratio of C:P was 15.6 for SAM coated surface and after the attachment of Paracetamol the ratio of C:N was 10.7. These ratios show the reduction in the atomic percentage of carbon after drug attachment since less carbon-rich Paracetamol contains 8 carbon atoms with 3 heteroatoms compared to 16-PhDA (which presents a carbon-rich surface due to a long alkyl group and buried phosphonate). However, there are possibilities for small errors since nitrogen is present in the atmosphere as a contaminant [35].

Due to the presence of nitrogen in Paracetamol, its concentration was noted to increase; however, this is not a large increase since the drug molecule contains only one nitrogen atom. The concentration of phosphorous (from 16-PhDA) and the underlying metals (titanium, aluminium and vanadium) were observed to decrease and this further confirms the functionalisation of SAMs with Paracetamol.

A very small contribution from chlorine and sulphur was observed after the attachment of Paracetamol and this is likely to be added from the SOCl_2 used in the intermediate step. Since the 16-PhDA SAMs have 2 hydroxyl groups ($-\text{OH}$) in each head group, there may be the possibility for the addition of chlorine or the sulphur to those hydroxyl ends to form $\text{Ti}-\text{O}-\text{P}-\text{OCl}$ -alkyl chain and/or $\text{Ti}-\text{O}-\text{P}-\text{S}$ -alkyl chain. Although the abovementioned reaction is possible, it depends only on the availability of a hydroxyl group for the reaction as both hydroxyl groups can bond to the Ti6Al4V surface initially before reaction of the SAMs.

Carbon contamination is unavoidable as it is present as a contaminant in the atmosphere. However, a small amount of nitrogen was also observed on the Ti6Al4V sample as a contaminant in both the control and SAM coated surfaces. The presence of these elements on the metal oxide surface as contaminants was reported in literature [23, 31]. Hence the ratio of carbon to the underlying metal composition of the surface has been used to determine the adsorption of SAMs and Paracetamol to the metal surface.

Fig. 8 shows the ratio of carbon and oxygen to the underlying metals (titanium + aluminium and vanadium) and the ratio of carbon to oxygen for all three samples (control, SAM attached and Paracetamol coated). It can be observed that the carbon and oxygen concentration increased after SAM attachment and functionalisation confirming the attachment of the Paracetamol to the tail group of 16-PhDA monolayers.

A comparison of the C 1s spectra obtained for the control, 16-PhDA SAM coated and SAMs functionalised with Paracetamol is shown in

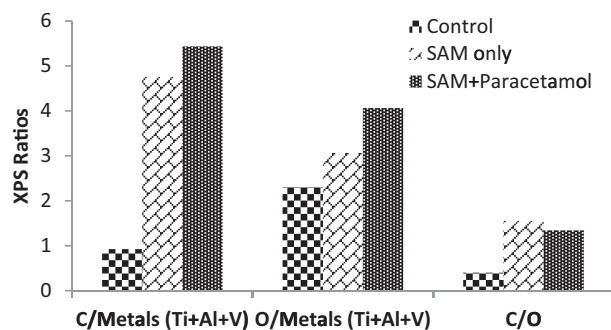


Fig. 8. Ratio of carbon and oxygen to its underlying metals and C/O ratio for control, SAM coated and Paracetamol coated surfaces.

Fig. 9. High-resolution spectrum of C 1s for the control sample was deconvoluted into two components. Peaks formed at 285.3 eV and 286.2 eV were assigned to C–C and C–O of hydrocarbon contaminants. Adsorption of hydrocarbon as contaminants to metal oxide surfaces has been discussed in literature and the major source for these contaminants is adventitious carbon in air, solvents (used for cleaning) and from hydrocarbons (containing proteins and oils) due to manual handling [35–38].

The C 1s spectrum for the SAM coated Ti6Al4V surface was deconvoluted into three components. The peaks observed at 284.9 eV, 286.5 eV and 289.3 eV were assigned to C–C, C–O and C=O [23]. A significant increase in the relative C–C peak intensity compared to the control surface was due to the 16 membered carbon chain $[\text{HOOC}(\text{CH}_2)_{15}\text{PO}(\text{OH})_2]$ in the 16-PhDA molecule. The C–O and C=O are also from the 16-PhDA molecule, present in the head and tail group of the chain. The significant change in the intensity of C–C, and C–O and the introduction of C=O peak confirms the modification of SLM fabricated Ti6Al4V surface with 16-PhDA monolayers.

On characterising the Ti6Al4V surface functionalised with Paracetamol, the deconvoluted C 1s spectrum rendered four components at 284.7 eV, 285.8 eV, 286.9 eV and 289.1 eV. These peaks can be assigned to C–C, C–O, C–N and C=O respectively [17,23]. Comparing this to the SAM coated surface, the peak intensity of C–C and C=O has changed. Also the introduction of C–N peak at 286.9 eV was observed. This C–N peak is from the amide group in Paracetamol.

Paracetamol powder in its pure form (as purchased) was characterised using XPS to obtain the spectrum for C 1s region (Fig. 9d). The deconvoluted spectrum showed the existence of four components C–C (284.7 eV), C–O (286 eV), C–N (288.2 eV) and C=O (291.2 eV) [23,39]. These peaks can be assigned to carbon atoms in hydrocarbon, hydroxyl, amide and ester groups of Paracetamol. The XPS results obtained for Paracetamol coated Ti6Al4V surface was in good agreement with the results obtained for the C 1s region of Paracetamol powder. However, a small change in the peak binding energies was noted and this could be due to errors in peak integration during deconvolution and might also be due to minor calibrating errors due to sample charging.

The O 1s region spectrum obtained using XPS for the control sample (Fig. 10a) was deconvoluted into two components, metal oxide at 530.5 eV and oxygen atoms in O–C at 532.1 eV [40–42]. The metal oxide peak observed at 530.5 eV is likely to be mostly due to titanium oxide; however, a small proportion of aluminium and vanadium oxides is also possible due to the presence of these elements in Ti6Al4V alloy. In agreement with the C 1s spectrum, the O 1s spectrum also showed the existence of O–C species on the control sample as contaminants.

On deconvoluting the O 1s spectrum for the 16PhDA SAM coated Ti6Al4V surface, three components were obtained (Fig. 10b). The peaks formed at 530.4 eV, 531.9 eV and 533.2 eV were assigned to metal oxides, O–C and O=C respectively [41–43]; however, there is also the possibility for a small contribution from aluminium oxide for the peak observed at 531 eV. The relative intensity of metal oxide peak at 530.4 eV for the SAM coated sample can be observed to decrease and the O=C peak at 533.2 eV to increase when compared to the control sample. The introduction of O=C at 533.2 eV should mostly derive from the carboxylic group at the terminal end of the 16-PhDA molecule. These observed changes compared with the control sample can be attributed to the adsorption of 16-PhDA monolayers on to the SLM fabricated Ti6Al4V surfaces.

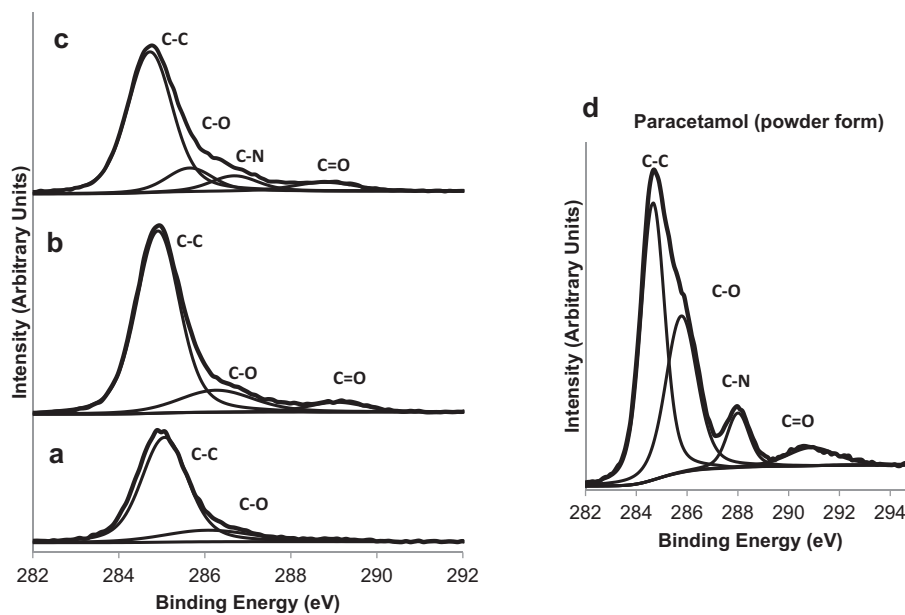


Fig. 9. High resolution spectra of carbon 1s region obtained for (a) control, (b) SAM attached, (c) Paracetamol coated samples, (d) shows the C 1s region for Paracetamol powder characterised using XPS.

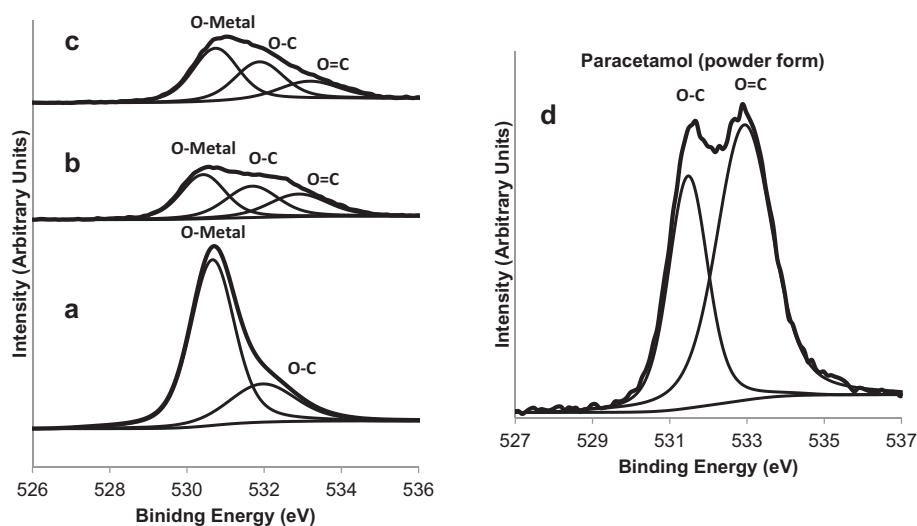


Fig. 10. XPS spectra obtained for the oxygen 1s region for (a) control, (b) SAM attached, (c) Paracetamol coated samples, (d) shows the O 1s region for Paracetamol powder characterised using XPS.

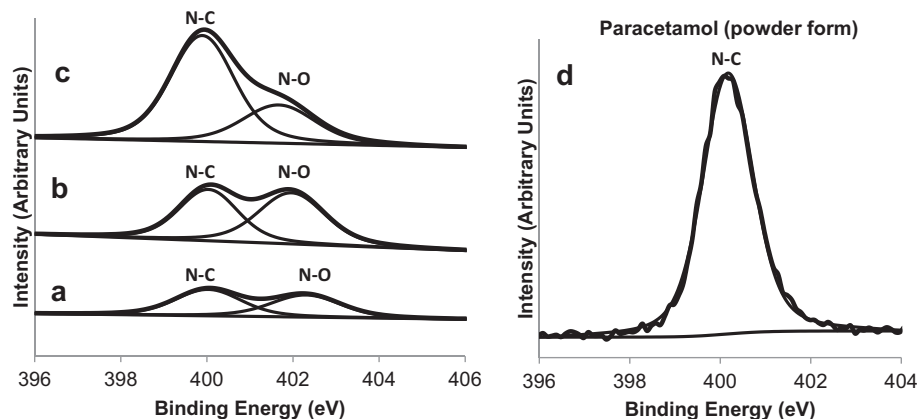


Fig. 11. N 1s region for (a) control, (b) SAM attached, (c) Paracetamol coated samples, (d) shows the N 1s region for Paracetamol powder characterised using XPS.

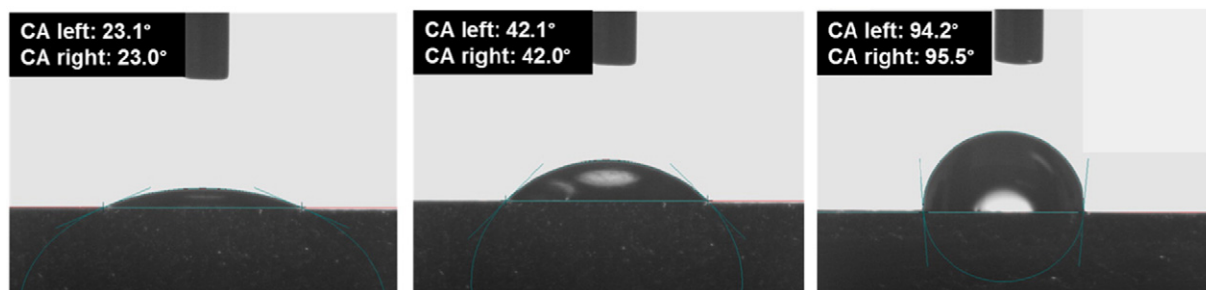


Fig. 12. Contact angles obtained for SLM fabricated Ti6Al4V surfaces after a) cleaning b) 16-PhDA SAM attachment, c) Paracetamol attachment.

After functionalising SAMs with Paracetamol, the deconvoluted O 1s XPS spectrum rendered three components metal oxide (530.8 eV), O–C (532.1 eV) and O=C (533.4 eV) and are shown in Fig. 10a. Although the O–C and O=C are expected only from Paracetamol, a small contribution from the underlying monolayers is possible. The XPS characterisation of Paracetamol powder for the O 1s region rendered two components including O–C (531.7 eV) and O=C (533.1 eV) on deconvolution [41] as shown in Fig. 10d. On comparing the deconvoluted O 1s peaks obtained for the Paracetamol coated surface with the O 1s peaks obtained for the Paracetamol powder, both the peaks were in good agreement. However, there was a slight variation in the binding energies. This further confirms the attachment of Paracetamol to the 16-PhDA adsorbed Ti6Al4V surface.

Deconvoluted N 1s spectrum showed the presence of N–C and N–O at 400.3 eV and 402.6 eV respectively on the control surface (Fig. 11a). Although nitrogen is not expected on the Ti6Al4V surface (as it is not in the composition), a small amount of nitrogen was observed. Similar to carbon, nitrogen also has strong affinity towards metal oxides and their presence on metal oxide surfaces could be from nitrogen containing carbon contaminants [23]. Similar to the control sample, the SAM coated surface also showed the existence of nitrogen at 400.1 eV (N–C) and 402.3 eV (N–O) after deconvolution (Fig. 11b) [37]. This could also be from contamination of the surface as the peaks were observed similar to the control samples. Furthermore, the 16-PhDA SAMs do not have nitrogen in their structure.

The deconvoluted N 1s spectrum for the Paracetamol coated Ti6Al4V surface also showed two components, N–C at 400.1 eV and N–O at 401.9 eV (Fig. 11c). Although a small contribution to these peak from contaminants is possible since the other samples (control and SAM coated) were shown to have contaminants, the N–C peak is distinct and increased in relative intensity. XPS characterisation of Paracetamol powder for the N 1s region showed a single N–C peak at 400.3 eV (Fig. 11d).

Wettability of a surface is influenced by its surface roughness, film thickness and its chemical composition/functional group [44]. The as-

fabricated SLM surface was rough and this may have affected the wettability. Hence a mechanically polished surface was used. Static contact angles obtained for the control, 16-PhDA SAM attached and Paracetamol coated surfaces are shown in Fig. 12. Fig. 13 shows the contact angle values obtained graphically. It can be observed that the control and 16-PhDA SAM deposited surfaces were highly wettable whereas the Paracetamol coated surface was more hydrophobic. The wettability of the surface cleaned control surface was due to the presence of metal oxides which impart a high surface energy. Wettability of the 16-PhDA SAM coated surfaces was due to the presence of carboxylic acid in their terminal group. Since the SAM coated surface was functionalised with Paracetamol, it would be expected that a methyl group ($-CH_3$) is exposed as the terminal group leading to a lower energy surface. In agreement with this, the measured contact angles showed the Paracetamol coated surface to be more hydrophobic with a contact angle higher than 90° . Thus, both the XPS characterisation and surface wettability measurements are consistent with the attachment of Paracetamol to the 16-PhDA SAMs and functionalisation of SLM fabricated Ti6Al4V surface with Paracetamol.

4. Conclusion

16-PhDA phosphonic acid SAMs were successfully attached to a SLM fabricated Ti6Al4V surface. The 16-PhDA SAMs were stable on the surface for more than 28 days when immersed in Tris-buffer solution before significant desorption from the surface was observed. Changes observed in the surface chemistry of the Ti6Al4V surface at each stage through XPS characterisation and surface wettability measurements are consistent with the functionalisation of monolayers with Paracetamol. This study showed the attachment and stability of 16-PhDA SAMs on SLM fabricated Ti6Al4V surface and the successful immobilisation of Paracetamol to 16-PhDA SAMs. However, nitrogen and carbon alone cannot be used as a key element to confirm the Paracetamol attachment, since they are present on the surfaces as a contaminant and may affect the result. Hence, Paracetamol may not be the ideal drug of choice to prove the immobilisation of the drug to SAMs. The use of drugs with other elements that are unlikely to be present in the atmosphere as contaminants could be used to prevent contamination affecting the results in future studies.

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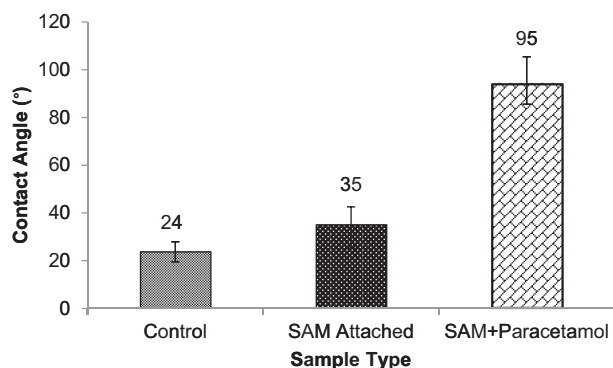
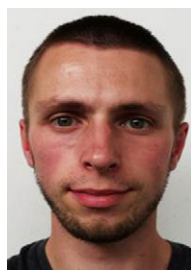


Fig. 13. Static water contact angle obtained for control, SAM coated and Paracetamol coated Ti6Al4V samples.

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