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A critical investigation of some of the procedures employed in the surgical use of titanium

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Rigorous and exhaustive procedures are employed when titanium (Ti) is surgically implanted, whether for orthopaedic or dental applications. Many of these are adopted because it is thought that surface cleanliness is paramount for clinical success. This paper critically examines the necessity for some of these procedures, concentrating on the surface chemistry of Ti plates. Radio frequency plasma treatments are used to remove contamination from "as received" Ti plates; XPS and ToF-SIMS were used to monitor the effects of surface chemistry. Ti plates are contaminated by immersing them in BSA or by deliberate contamination with the endospores of *Bacillus stearothermophilus* ATCC 7953. The effectiveness of simple cleaning procedures to remove BSA/*Bacillus stearothermophilus* are investigated. Attention is given to both the surface cleanliness and sterility after cleaning.

1. Introduction

In part one of this study [1], the surfaces of titanium (Ti) plates, as models for Ti dental implants, were characterized by X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Ti plates were prepared with two topographies: rough and smooth. XPS data were used to construct a model of the surface chemical structure of the plates, from the gas–solid interface through to pure Ti metal. At the surface of the "as-received" plates, which had undergone the same preparative procedure as an implant, considerable surface contamination was detected, in particular, high levels of carbon (C). The nature of this C was elucidated by fitting the C 1s core line and from the SIMS analysis. The results revealed that the topmost surface consisted of a patched hydrocarbon overlayer, rich in C–O functionalities; SIMS analysis showed this overlayer to be a mixture of PDMS, fatty acids and amides. Ti metal, as well as forming a surface oxide layer, was shown to have reacted with atmospheric water, and subsequently hydroxylation had occurred, as witnessed in the O 1s core line. On the smooth side of the plates, the TiO_2 layer was found to be $\sim$2.0 nm; as revealed by the detection of Ti metal. In addition, XPS detected other elements, and while of lower concentration, their importance ought not to be ignored.

The data reported were considered of particular interest in view of the considerable care taken in the surgical use of Ti implants. Emphasis is put on avoiding contact with other materials, and immediate disposal is recommended if Ti becomes in any way contaminated (for example, see [2]). Rigorous and exhaustive procedures are employed when Ti is surgically implanted, because it is thought that maintaining the surface cleanliness of the Ti is paramount [3–5]. While these precautions may well be important in ensuring the success of the implant, the rôle (if any) and fate of surface contaminants (hydrocarbons, N, Ca, Na, S, P, Si, K, Pb, and Fe) during osseointegration is unknown.

This paper concentrates on the surface chemistry of Ti implants. We report on the use of XPS and ToF-SIMS to investigate further the nature of surface contaminants, and we report on the effectiveness of two simple cleaning procedures in removing contamination from "as-received" plates. These procedures are also tested to remove deliberate contamination of BSA and *Bacillus stearothermophilus* ATCC 7953 from Ti surfaces.

2. Experimental procedures

2.1. Materials

Ti plates were supplied by Nobelpharma. The machining of the Ti plates (surface area approximately 3 cm × 1 cm) was carried out in such a manner that the rough side approximated the surface microstructure of an implant fixture. Cleaning and sterilization based on that used by the manufacturer was followed.

2.2. Spectral measurements

XP spectra were recorded on a VG Scientific spectrometer using MgK_α X-rays. The plates were fixed on an aluminium stub using brass clamps. Measurements

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were made at a "take-off" angle of 30° with respect to the sample surface.

XPS data manipulations (i.e. background subtraction's (linear type), measurements of peak area ratios, etc.) were carried out on a VGS 5000 data system using VGS software. In order to determine surface compositions from XPS data, accurate sensitivity factors (sfs) are required to convert peak area ratios into atomic percentage ratios. In this work the sfs of Ward and Wood [6] were employed.

ToF-SIMS measurements were made on a VG Ionex ToF-SIMS instrument using a pulsed liquid metal primary ion source. Positive SIM spectra were obtained, keeping the ion dose below the threshold of the static regime (10^{13} ions/cm^2).

2.3. Plasma treatments

The radio-frequency (RF) plasma reactor was manufactured by Chemex, Co. Durham, UK. The glass reactor (10 cm in diameter and 20 cm long) was always operated under the same conditions: plasma power of 200 W, reactor process pressure of 27 Pa and for a period of 3 min.

2.4. Bovine serum albumin (BSA) contamination and cleaning

Contamination was achieved by soaking the samples in a solution of BSA for 1 s. This protein had been supplied by Sigma (A-6793) and was prepared at a concentration of 70 mg/ml in sterile H_2O, this concentration being close to that of human albumin in blood. The two cleaning procedures were carried out consecutively. First the samples were cleaned with a nylon brush using detergent ("Micro" solution; International Products Corporation, Chislehurst, Kent, UK) and copious amounts of water, rinsed with deionized water, boiled in 1% (v/v) "Micro" solution in deionized water, and finally rinsed with deionized water before drying in air. After this rather simple cleaning procedure 1, samples were analysed by XPS. Selected samples, after procedure 1, underwent Argon plasma etching (under the same conditions mentioned in Section 2.3), in order to clarify procedures this second treatment being the cleaning procedure 2.

2.5. Bacterial contamination and cleaning

Free endospores of *Bacillus stearothermophilus* ATCC 7953 were prepared by growing cells in a minimal salts medium containing a low concentration of glucose (0.4 g/l) to encourage sporulation. Spores were harvested by centrifugation and suspended in water or in sterile horse blood and adjusted to a concentration of 10^7 spores/ml using a Helber counting chamber. Aliquots (10 μl; 10^5 spores) of each suspension were then applied to the titanium pieces and dried at 37 °C overnight. In each experiment, at least one Ti piece was left uncontaminated, to check for sterility, and two pieces that had been inoculated with spores were cultured directly to check for spore viability.

Ti pieces were first cleaned using the two procedures described above for BSA, but subsequently procedure 1 was modified to involve only very light brushing with either detergent (1% (v/v) "Micro" solution) followed by rinsing with deionized water/or with deionized water only. The efficiency of each cleaning procedure was tested by subsequently placing each piece in Spore Strip Broth (Oxoid) and incubating at 55 °C for up to 7 days.

3. Results and discussion

3.1. The "as-received" plates

The "as-received" Ti plates displayed considerable surface hydrocarbon contamination (Table I). Typically, on a plate's surface, 43–45 at % C was found; this figure excludes hydrogen (not detected by XPS). If this hydrocarbon was present as a continuous overlayer, this amount would correspond to an overlayer approximately 0.9 nm thick.

A detailed description of the Ti plates as elucidated by XPS and SIMS has previously been reported by the authors [1].

3.2. The "as-received" plates after glow-discharge argon plasma cleaning

The argon plasma treatment reduced the amount of carbon contamination detected by over 50% (Table II). Cleaning was found to be more efficient on the smooth side of the Ti plates. The shape of the C 1s core line (smooth and rough sides) was considerably changed; the remaining carbon was found to be mostly C–C and C–H at a binding energy of 285 eV; the other peaks at chemical shifts + 1.4, + 2.9, and + 4.2 eV were substantially reduced, these peaks corresponding to C–O, C=O, O–C=O environments respectively (Fig. 1).

From the shape of the O 1s core line (Fig. 2) it is evident that after argon plasma treatment O was still present in at least two different chemical environments, the dominant peak (at binding energy 530 eV) can be assigned to TiO_2. The loss of C–O, C=O, and O–C=O functionalities manifested as a reduction in the intensity of the high binding energy shoulder. The

TABLE I Atomic percentage of elements found in the "as-received" Ti plates, when analysed by XPS

Elements of core level	As-received; rough side (%)	As-received; smooth side (%)
C 1s	42.32	43.92
O 1s	36.89	39.83
Ti 2p	11.57	09.82
N 1s	06.02	04.30
Ca 2p	00.45	Trace
Na 1s	01.15	01.52
S 2P	None	None
P 2P	Trace	00.63
Si 2P	00.99	None
Pb 4f	00.60	00.27

TABLE II Atomic percentage of elements found in the "as-received" Ti plates after cleaning with Ar plasma, when analysed by XPS

Elements of core level	As-received; + Ar plasma; rough side	As-received; + Ar plasma smooth side
C 1s	17.77	18.63
O 1s	55.96	56.05
Ti 2p	21.05	20.78
N 1s	00.70	00.88
Ca 2p	00.32	00.29
Na 1s	01.08	00.75
S 2P	02.33	01.52
P 2P	Trace	00.64
Si 2P	00.03	Trace
Pb 4f	00.47	00.27
K 2P	00.39	00.19

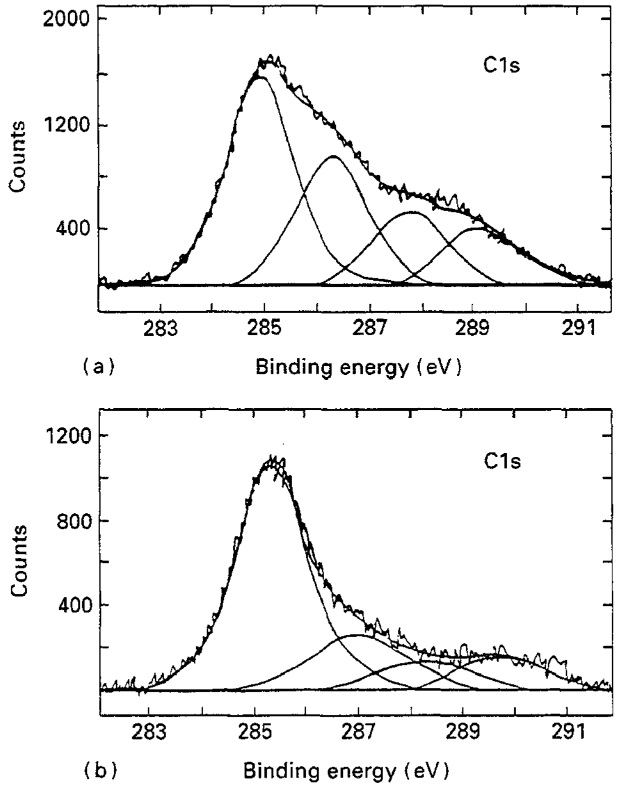

Figure 1 The XPS C 1s core level of "as-received" Ti plates (a) before and (b) after Ar plasma cleaning of surfaces.

cleaner surface allows for direct analysis of the Ti-oxide layer; the surface Ti : O ratio has been calculated as 1 : 2.66, but a small signal from underlying pure Ti was observed in the Ti core line, indicating that the O content of the oxide layer was perhaps greater than this ratio suggests. However, the broadness of the shoulder is consistent with a multiplicity of O functionalities, almost certainly some of which arise from different forms of chemisorbed and physisorbed water on or within the TiO_2 surface layer [7]. A more detailed analysis of this signal is prevented by the resolution of the spectrum and the large number of possible O environments.

The centre of the Ti 2p1/2 was detected at ~458 eV consistent with the chemical environment TiO_2. The Ti signal intensities detected on the smooth and rough sides were doubled (Tables I and II). These results strongly suggest that after plasma treatments the surfaces were relatively free from large amounts of hydrocarbon and O-rich carbon contaminants. In addition, the N 1s core level signal was drastically reduced by the plasma cleaning process.

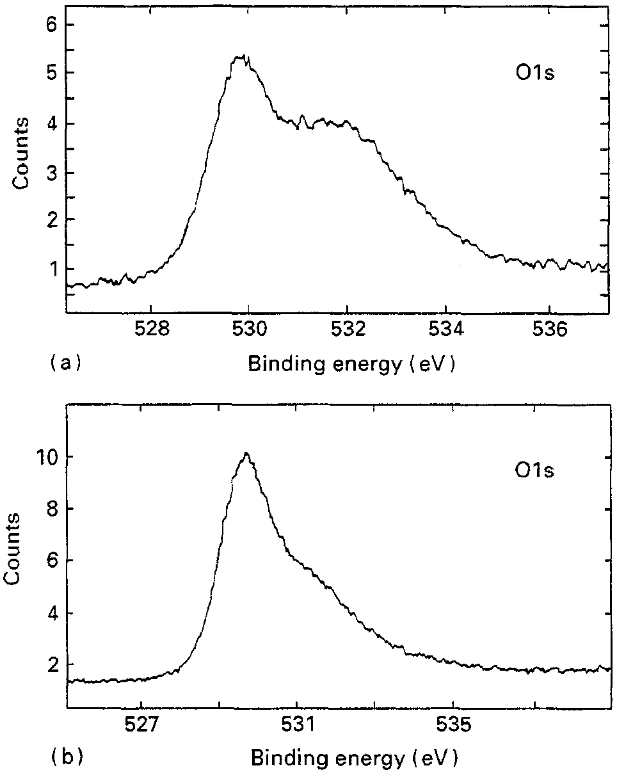

Figure 2 The XPS O 1s core level of "as-received" Ti plates (a) before and (b) after Ar plasma cleaning of surfaces.

Low concentrations of other elements were detected in the XPS analyses (Table II). The amounts differ from those detected in the "as-received" plates (Table I); however, for most elements, because of the low concentrations monitored, it is difficult to determine whether the variations are outside those of experimental error. The appearance of potassium (K) and sulphur (S) is difficult to explain; polymers treated in the plasma apparatus do not show detectable levels of these elements and, therefore, it is certain that these elements did not arise from the plasma treatment (i.e. from the glass wall of the plasma vessel). We believe these elements must have been present on the "as-received" plates, but their signals were masked by the hydrocarbon.

The outermost surface of the Ti plates (0.8–0.9 nm) were probed by SIMS. The positive ions of the Ti samples after Ar plasma treatment proved most informative in comparison with the "as-received" plates, the most important observations were:

(1) The absence of ions assigned to the series of: amides, for example the ion at $m/z = 256$; polydimethylsiloxane (PDMS), for example the ions at $m/z = 207$, 221 and 281; and fatty acids [1]. (However, the negative ion spectra show low levels of the latter series.)

(2) As a consequence of (1), the major ions diagnostic of TiO_2, at $m/z = 64$ (TiO^+), 65 ($TiOH^+$), and 81 ($TiO.OH^+$) were detected, in addition to the signal

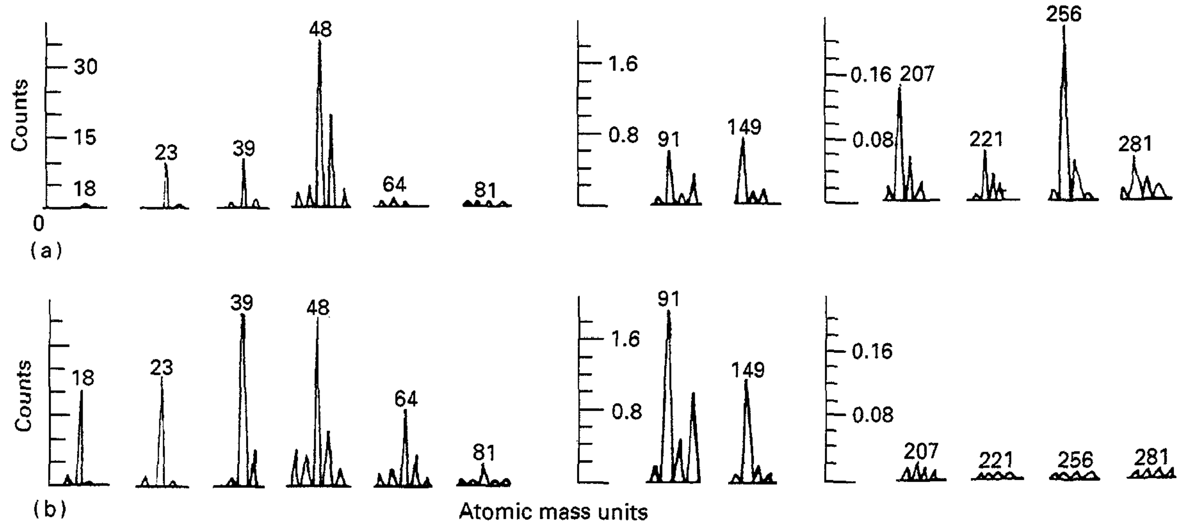

Figure 3 The positive ToF-SIM spectrum of the surface of a Ti plate (a) before and (b) after Ar plasma treatment.

from Ti^+ which was enhanced and detected again with ease at $m/z = 48$.

(3) At low mass, the detection of an ion at $m/z = 18$ is most likely to be water, since after the Ar plasma treatment the surface is highly reactive with the atmospheric gases. This is supported by the shape of the O 1s core level (Fig. 2 and Table II), which shows that there was excess of O not associated with TiO_2. The peak at $m/z = 23$ assigned to Na^+ was introduced by the "Micro" solution. The peak at $m/z = 39$ is assigned to K^+.

(4) Polymeric surfaces bombarded with ions are known to degrade forming aromatic compounds. This could explain, for example, the enhancement in the signals at $m/z = 91$ $(C_7H_7)^+$ and 149 assigned to the phthalate ion $(C_8O_3H_5)^+$. The SIMS negative ion spectra was not informative.

3.3. Contamination by BSA and cleaning

After contamination the XPS wide scan of the Ti plate surface reveals the disappearance of Ti (Fig. 4). Optical microscopic measurements revealed that the adsorbed BSA formed a layer 4 μm thick on the Ti surface. The four main elements were Ti, O, C and N (Table III).

Table III reports data on the composition of the BSA contaminated surface, and the results of applying cleaning procedures 1 and 2, with and without sterilization. The results show that cleaning procedure 1 alone had not removed all the contamination. However, the detection of a relatively significant Ti 2p signal indicates that the procedure had been very effective in reducing the BSA layer. However, when cleaning procedure 1 was followed by argon plasma treatment, the level of carbon contamination was reduced by a further 50%, this was coupled with a reduction in the signal from the N 1s core line, and an increase in the signals from O 1s and Ti 2p core lines. The data suggests that procedures 1 and 2 have produced a mainly TiO_2 surface with a low-level contamination.

A significant increase in the carbon level was introduced by autoclave sterilization. This leads us to suggest that by finding an alternative method of sterilization, such as argon plasma, a cleaner surface can be obtained, which may aid the osseointegration process and the long-term integrity of the implant. However, more work is needed on the use of argon plasma for sterilization, and the effect of the surface contaminants on the osseointegration process.

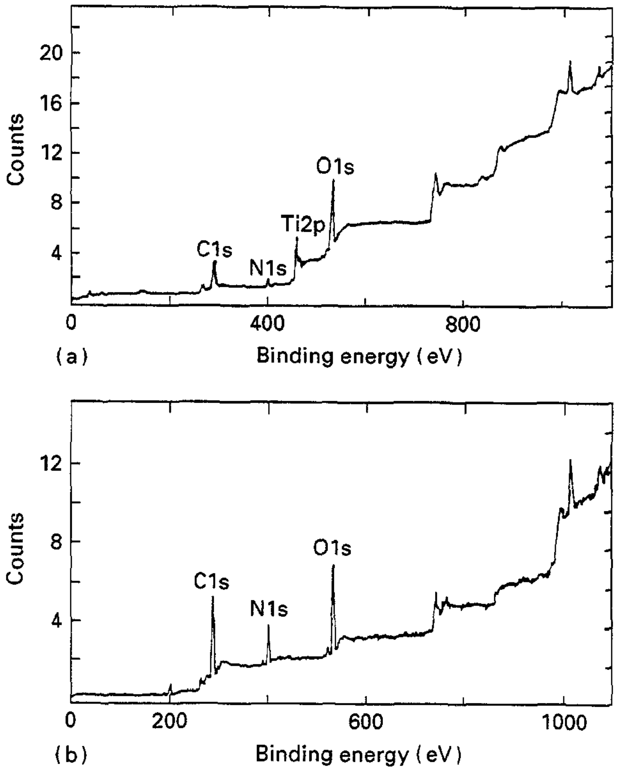

Figure 4 The XPS wide scans of the Ti plates (a) before and (b) after BSA contamination.

3.4. Bacterial contamination and cleaning

Generally, it is accepted that for successful eradication of organisms by whatever sterilization process, as much organic matter as possible must be removed. Therefore, contaminating the titanium samples with bacterial endospores in the presence of blood posed

TABLE III Quantified XPS data from the BSA-contaminated Ti plates before and after cleaning

Elements of core level	After contamination with BSA (%)	Contaminated with BSA and cleaned using procedure (1) (%)	Contaminated with BSA and cleaned using procedures (1) and (2) (%)	Contaminated with BSA, cleaned using procedures (1) and (2) and sterilized (%)
Ti 2p	00.00	20.01	22.41	17.59
O 1s	17.67	45.13	62.71	43.22
C 1s	68.41	31.39	13.75	37.86
N 1s	13.90	03.46	01.13	01.34

TABLE IV The effectiveness of cleaning procedures used to remove bacterial spores from the Ti plates

Cleaning/sterilization	Presence of spores
No cleaning	(+)
Proc. 1[a]	(−)
Proc.1 + autoclave	(−)
Proc.1 + Ar plasma	(−)
Detergent[b]	(+/−)[c]
Detergent + autoclave	(−)
Detergent + Ar plasma	(−)
Water	(+)
Water + Ar plasma	(+/−)

[a] Procedure 1: cleaned with nylon brush and detergent (1% (v/v) "Micro" solution), rinsed with deionized water, boiled in 1% (v/v) "Micro" solution in deionized water, and finally rinsed with deionized water before drying in air. [b] Cleaned with very light brushing and detergent (1% (v/v) "Micro" solution) and rinsed in deionized water before drying in air. [c] At least one replicate positive, some negative.

a significant sterilization challenge. Despite this, cleaning procedure 1 was very effective at removing the dried spores and blood from the titanium surface but alternative, gentler methods did not (Table IV). When argon plasma was used to supplement the cleaning, improved removal was obtained, and Ti samples in an experiment were rendered sterile or at least one of the replicates was sterile. However, success of the latter was reliant upon at least reducing the organic matter by the washing process because argon plasma alone could not remove all the material that had been applied to the titanium.

4. Conclusions

The surface chemistry of the "as-received" Ti plates, and by implication Ti implants, is more complex than has been previously reported [1, 8]. The topmost surface consists of a patched hydrocarbon overlayer, rich in O–C functionalities; SIMS analysis reveals this overlayer to be a mixture of PDMS, fatty acids and amides. Most of this layer was removed by a glow-discharge argon plasma cleaning treatment. From the XPS analyses, other elements were detected; while of lower concentration, their importance ought not to be ignored.

Two cleaning procedures were carried out to remove the deliberate contaminations of BSA and *Bacillus stearothermophilus* ATCC 7953 from the Ti plates. XPS results revealed that the cleaned surface consisted of mainly TiO_2. Simple washing with detergent and a nylon brush followed by argon plasma treatment was particularly effective at removing the BSA. In the case of the bacterial contamination, the results showed that complete removal of this type of contamination is possible.

It is not the intention of this work to provide a definitive cleaning method for Ti implants (to enable re-use etc.), rather, we show that from a surface chemical perspective Ti plates may be returned to their original state (clean and/or sterile), by simple procedures. However, the effect of cleanliness on osseointegration, and the long-term integrity of implants is still a matter for conjecture.

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