Si+ ion implantation reduces the bacterial accumulation on the Ti6Al4V surface

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Abstract. Ti6Al4V is one of the most commonly used biomaterials in orthopedic applications due to its interesting mechanical properties and reasonable biocompatibility. Nevertheless, after the implantation, microbial adhesion to its surface can provoke severe health problems associated to the development of biofilms and subsequent infectious processes. This work shows a modification of the Ti6Al4V surface by Si+ ion implantation which reduces the bacterial accumulation under shear forces. Results have shown that the number of bacteria remaining on the surface at the end of the adhesion experiments decreased for silicon-treated surface. In general, the new surface also behaved as less adhesive under in vitro flow conditions. Since no changes are observed in the electrical characteristics between the control and implanted samples, differences are likely related to small changes observed in hydrophobicity.

1. Introduction
Bacteria prefer a community-based surface-bound, sedentary lifestyle to a nomadic existence. There may be an obvious explanation for bacterial adhesion, because nutrients in an aqueous environment tend to concentrate near a solid surface [1].

As an oversimplified rule of thumb, primary adhesion between bacteria and abiotic surfaces is generally mediated by nonspecific interactions, whereas adhesion to living or devitalized tissue is accomplished through specific molecular (lectin, ligand or adhesion) docking mechanisms. Primary adhesion is reversible and defines the further adhesion between the bacterial cell surface and the conditioned surface of interest. Once the microorganisms reach critical proximity to the surface, the final determination of adhesion depends on the net sum of attractive or repulsive forces generated between both surfaces, including electrostatic and hydrophobic interactions [2].

The ability to control microbial adhesion is of enormous importance in healthcare, particularly in modern surgery where postoperative implant-associated infections are still an unresolved and serious complication. This challenge is compounded by the ever-increasing problem of antibiotic resistant hospital and community acquired infections [3, 4]. For this reason new strategies are focused on minimizing microbial surface colonization by modifying the biomaterial surface and antibacterial coatings of Ti6Al4V have been recently proposed by different research groups [5-7].
In this line, this work shows a new modification of the Ti6Al4V surface by Si+ ion implantation which reduces the bacterial accumulation under in vitro flow conditions. Surface characterization is carried out by means of hydrophobicity and isoelectric point.

2. Materials and methods

2.1. Ti6Al4V surface modification.
Ion implantation was carried out by using a F4Si precursor on the surface of 20 mm diameter disks of Ti6Al4V kindly supplied by Surgival SA, Spain. Ti6Al4V without implantation will be denoted as “control” and Ti6Al4V implanted will be denoted as “implanted”.

2.2. Surface characterization.
2.2.1. Hydrophobicity: It was quantified through the water contact angle ($\theta_W$) on the sample surface.
2.2.2. Surface Gibbs Energy: It was evaluated from the contact angles of water, formamide ($\theta_F$) and diiodomethane ($\theta_D$) by applying the Young Equation [8] and Van Oss approach [9]. The surface Gibbs energy ($\gamma_{\text{total}}$) was the sum of the Lifshitz-van der Waals component ($\gamma_{\text{LW}}$) and acid-base component ($\gamma_{\text{AB}}$) which, in turn, is the geometric mean of the electron-donor ($\gamma^-$) and electron-acceptor ($\gamma^+$) parameters.
2.2.3. Isoelectric point: zeta potential ($\zeta$) was measured as pH function and isoelectric point (IEP) was obtained as the pH at which $\zeta$ was zero [10].

2.3. Bacterial strains.
Three gram-positive strains with different EPS-production were used: Staphylococcus aureus ATCC29213 (S. aureus), Staphylococcus epidermidis ATCC35984 (S. epidermidis4), Staphylococcus epidermidis HAM892 (S. epidermidis2).

2.4. Adhesion experiments.
Bacterial adhesion experiments were carried out at 37 °C in a parallel plate flow chamber and the results were analysed in terms of flow or static conditions by selecting a constant flow rate or stopping the bacterial flow. Different protocols were followed:
2.4.1. Dynamic adhesion: the flow chamber was placed so that the alloy surface was on the top side of the flow channel and the bacteria were allowed to attach while flowing at a constant rate for 20 min. The number of bacteria at the end the experiments dynamic was denoted by $N_{D-20\text{min}}$. It was also analysed the initial adhesion rate to the surface ($j_D$).
2.4.2. Static adhesion: the sample was placed on the bottom side of the flow channel, the flow was stopped and the bacteria were left to deposit on the surface for different time interval periods. Static adhesion rates ($j_S$) were obtained and compared to that of dynamic.
2.4.3. Shear forces: At the end of the experiments two consecutive air-liquid interfaces were passed through the flow channel and the number of bacteria remaining on the surface were analysed in order to check the strength of the bacterial retention.

3. Results and discussion
Table 1 shows the surface characterization of both control and implanted samples. There are small changes in contact angles but the implanted surface is slightly more hydrophilic than the control. This also implies a higher polarity of the treated surface since the polar liquids (W, F) present lower contact angles and $\gamma_{\text{AB}}$ is higher. There is no difference between samples in the IEP within experimental error.

Figure 1 presents interesting information from the bacterial adhesion tests. It is observed that initial adhesion rates to control and implanted are not statistically different (Fig. 1a). Only in the case of S. epidermidis2 a decrease in $j_D$ is observed in the implanted sample respect to control. This strain also
presents the highest decrease in the final number of adhered bacteria after dynamic experiments (Fig. 1b). N_D-20min also diminishes for _S. aureus_ in the implanted. Information provided by static experiments are different to that of dynamic, as showed when comparing Figs 1a and 1c. j_S is always higher than j_D, indicating that static adhesion is highly influenced by sedimentation processes. Relationships between control and implanted in both experiments are also different. In the case of static adhesion Si+ ion implantation never reduces adhesion (Fig. 1c) on the contrary; it is increased for both strains of _S. epidermidis_. An interesting observation is that after the passage of two air-liquid interfaces the bacterial detachment is more effective in silicon-treated surface than in control (Fig. 1d), which could enhance the applicability of this technique in those orthopaedic applications in which shear forces are present.

**Table 1.** Contact angles of water (θ_W), formamide (θ_F) and diiodomethane (θ_D), Lifshitz-Van der Waals (γ_W^L) and acid-base (γ_AB) components as well as electron-acceptor (γ^+) and electron-donor (γ^-) parameters and the total surface Gibbs energy (γ^Total) for control and implanted samples.Isoelectric points (IEP) are also represented.

| Contact Angle | Surface Gibbs Energy | IEP |
|---------------|----------------------|-----|
| θ_W | θ_F | θ_D | γ_W^L | γ_AB | γ^+ | γ^- | γ^Total | IEP |
| **Control** | | | | | | | | |
| 58.6 | 48.7 | 40.6 | 32.4 | 8.1 | 0.7 | 24 | 40.5 | 5.5 |
| ±1.8 | ±1.6 | ±1.4 | ±0.7 | ±1.7 | ±0.2 | ±3 | ±2.4 | ±0.9 |
| 53.5 | 42.5 | 38.3 | 33.1 | 10.9 | 1.14 | 26.1 | 44.0 | 4.8 |
| ±0.7 | ±0.7 | ±0.7 | ±0.6 | ±0.8 | ±0.11 | ±1.1 | ±1.4 | ±0.8 |
| **Implanted** | | | | | | | | |

**Figure 1.** Bacterial adhesion experiments. j_D: initial dynamic adhesion rate locating the sample at the top of the flow channel (a). N_D-20min: number of adhered bacteria at the end of the dynamic adhesion experiment (b). j_S: static adhesion rate locating the sample at the bottom of the flow channel (c). Percentage of remaining bacteria after passing two air-liquid interfaces (d).

Relationships between physico-chemical surface parameters and bacterial adhesion have been extensively studied [2, 7, 11]. However it is difficult to establish the exact contribution of each
magnitude to any particular adhesion process involving microorganisms. Electrostatic interactions tend to favor repulsion, because most bacteria and inert surfaces are negatively charged. *Stenotrophomonas maltophilia* is one exception to this rule, and the overall positive surface charge of this organism, at physiological pH can promote primary adhesion to negatively charged materials such as Teflon [12]. In our case, the IEP obtained for both surfaces indicates that they are negatively charged at physiological pH (about pH=7). As indicated by different research groups, hydrophobic-hydrophilic interactions probably have greater influence on the outcome of primary adhesion [7, 13, 14]. In the present research the small changes in the hydrophobicity of the surface after implantation seems to be related to the lower retention of the three strains (Fig. 1d). Silicon-treated surface become more hydrophilic and this means a higher affinity for water under shear conditions, reducing the final bacterial accumulation on the surface.

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