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Rodriguez-Sanchez, J., Gallardo-Moreno, A.M., Bruque, J.M. et al. (1 more author) (2016) Adsorption of human fibrinogen and albumin onto hydrophobic and hydrophilic Ti6Al4V powder. Applied Surface Science, 376. pp. 269-275. ISSN 0169-4332

https://doi.org/10.1016/j.apsusc.2016.03.014

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Adsorption of human fibrinogen and albumin onto hydrophobic and hydrophilic Ti6Al4V powder.

Jesús Rodríguez-Sánchez, Amparo M. Gallardo-Moreno, José M. Bruque, M. Luisa González-Martín*.

Department of Applied Physics, Faculty of Science, University of Extremadura and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Avda de Elvas s/n, 06006 Badajoz, Spain.

Corresponding author: E-mail address: jrs@unex.es (Jesús Rodríguez-Sánchez)

Abstract

Adsorption of proteins on solid surfaces has been widely studied because of its importance in various biotechnological, medical and technical applications, such as medical implants or biosensors. One of the main problems is the adsorption-induced conformational changes because they often modify the biological activity of the proteins, which is believed to be a key factor on the subsequent cellular adhesion. The aim of this work is the study of the adsorption of human fibrinogen (Fg) and human serum albumin (HSA) onto Ti6Al4V particles, commercially available on different size, that are used to elaborate scaffolds to provide structural support to cell proliferation, promoting tissue development and bone regeneration among others. The study was done through the analysis of the adsorption isotherms and the electrical characterization of surfaces after adsorption in terms of the zeta potential (ζ). From this analysis it seems that Fg adsorbs preferentially vertically oriented (end-on) and HSA moves sequentially over the surface of the Ti6Al4V particles through dimmer formation, allowing adsorption progress over this initial bilayer. The zeta potential values of both proteins remain constant when the monolayer is formed. The study also extends the analysis of both adsorption behaviour and ζ potential characterization factors to the influence of the substrate hydrophobicity as this property can be modified for the Ti6Al4V by irradiating it with ultraviolet light (UV-C) without changes on its chemical composition[1,2].Differences at low protein concentrations were found for both isotherms and zeta-potential values.

Keywords: Ti6Al4VPowder; Adsorption Isotherms; Proteins; Zeta Potential; Hydrophobicity; Pulvimetallurgy.

1. Introduction

Ti6Al4V is the metallic material most commonly used for manufacturing prostheses because of its acceptable bioinertness ,mechanical properties[1]. Prostheses used to be built by turning extruded bars of the alloy but nowadays there is an increasing interest on implementing 3D printing techniques to this purpose. Additive manufacturing with powdered alloy allows fabrication of custom-made prostheses but also the design of scaffolds that combine a very well designed geometry but maintaining properties of the alloy. In this sense, an important advantage of metallic scaffolds against ceramic or polymeric scaffolds is their utmost mechanical properties, which is of paramount interest for repairing large bone defects. Despite a melting process among metallic powders allows the building of the construct, its external surface exposes the last layer of the powder particles used in printing to the physiological media.
In general, the final properties of any alloy as Ti6Al4V are highly conditioned by its fabrication procedure. For instance, temperature reached in the extrusion process, rate of cooling and diameter of the produced bar and atmosphere, among others factors, are able to alter the microstructure and the surface features of the alloy by modification of the oxides proportion and/or its surface microcrystallinity[2,3]. Since metallic powders are generally prepared by a very fast solidification process of melted droplets of the alloy produced in a gas or aqueous ambient, it is expected that the surface properties of the atomized Ti6Al4V powder would be different to those of the alloy obtained by extrusion or others procedures. Considering that the most external surface of the biomedical devices will come into contact with the physiological media, a good knowledge of their surface properties is needed.

The first event which occurs by contacting prostheses with physiological fluids is the formation of a precursor layer of adsorbed proteins on its surface[4]. The characteristics of that layer are crucial for the adequate behavior of the material of the implanted devices. This layer modifies the properties of the underlying material, making the surface friendlier to the biological surrounding. One of those properties is the surface electric charge, which is considered one of the main factors involved in the biological evolution of tissues around an implant[5] and that is modified accordingly by the adsorption of proteins. Because of the difficulties of measuring surface charge, the zeta potential, ζ, that indicates the potential between the Stern layer and the diffuse layer and that can be determined by several methods such as electrophoresis or streaming potential[6,7], is the most widely referenced property used to characterize such evolution.

Two of the most widely found proteins in human plasma are fibrinogen (Fg) and albumin (HSA). Fibrinogen is a soluble structural protein. It is present in blood plasma at an approximate concentration of 4.2 mg ml^{-1}. The fibrinogen molecule (340 kDa)[8] is highly anisotropic, with a ratio length/width greater than10[9], and shows a geometry that is depending on its degree of folding. Fibrinogen is precursor of fibrin[10] and fundamental in blood coagulation. Albumin (67 kDa)[11] is the most abundant protein in human blood. The total amount present in an adult is being approximately 300 g, nearly over 60% of the total protein content[12], at an approximate concentration of 35-54 mg ml^{-1}. Its structure, relatively simple, is defined as a blend of three homologous domains acquiring a heart shape, and in turn this assembly tends to form dimmers with each other[13]. Albumin plays an important role in the transport of fatty acids, hormones, metabolites and multiple drugs to several parts of the human body[12] and in keeping the osmotic pressure. Thus, how these two proteins behave when they are adsorbed on any biomaterial is of

great importance. In this line, there are several studies of fibrinogen and albumin adsorption on Ti6Al4V obtained from extruded bars[14,15], on Tic.p. as well as on TiO_2[16–20].

In previous works we proved that after irradiation with an UV-C source, Ti6Al4V discs taken from an extruded bar changes considerably its surface free energy [2,21] and also modifies the bacterial adhesion rate and viability on the alloy [21,22]. This valuable property affects the hydrophobicity of the surface that would modify the interaction of the surface with the surrounding proteins and in turn their adsorption behavior[23].

Therefore, we are aimed in this paper to the analysis of the adsorption of human fibrinogen and albumin on the surface of micron sized spherical particles of Ti6Al4V used for additive manufacturing of implants, on the base of their adsorption isotherms and the zeta potential evolution. Also, we will consider as a key parameter for the study the change of the hydrophobicitiy produced by the irradiation with UV.

2. Materials and methods

2.1. Substrate characterization

Spherical powder of Ti6Al4V (Goodfellow Cambridge Limited) was selected as adsorbent. According to manufacturer, these particles were processed by atomization in liquid state and then air-cooled for solidification. Also discs of Ti6Al4V cut from bars produced by extrusion were kindly provided by Surgival, Spain, and purchased to DKSH, Switzerland .

Surface composition was analyzed by X-ray Photoelectron Spectroscopy (XPS), with a K-Alpha (Thermo, UK), using an Al-Kα monochromatic X-ray source, with a spot size of 300 µm. The atomic percentages of elements were calculated using software and atomic sensitivity factors included with the instrument data system. Table 1 summarizes the values for the most relevant elements in powder and flat discs of Ti6Al4V. Residual traces of other elements were located, but in all cases at a rate < 2%. Also, relative values of aluminium and vanadium in respect to titanium, Al/Ti, V/Ti, and between aluminium and vanadium, Al/V, have been included in Table 1 to compare powder and discs surface composition. The XPS analysis revealed that the surface of the Ti6Al4V is mainly composed by Ti in the form of oxides, probably as TiO_2. It is noticeable the high percentage of Al for Ti6Al4V powder sample in respect to discs samples. This distinctive feature is clearly shown by the relative values of aluminium respect to titanium, Al/Ti, and to vanadium, Al/V, that are more than four times higher for powder than for flat samples, verifying the differences in surface composition between powdered and extruded samples.

Specific surface area, A_{sp}, was obtained by nitrogen adsorption isotherms at 77 K with an Autosorb AS-1 Series, (Quantachrome Instruments). Prior to the assays, the sample (0.5 g) was out-gassed during 12 hours at 300 °C. The average specific area obtained using the Brunauer, Emmet and Teller (BET) equation was 0.37 ± 0.05 $m^2 g^{-1}$, after three independent determinations.

The morphology of the Ti6Al4V particles was determined by Scanning Electron Microscopy (SEM) (Quanta 3D FEG, FEI Company, EE.UU.) using a secondary electron detector at 5 kV as accelerating potential. SEM image (Fig. 1) shows perfectly spherical non-porous particles.

Particle size distribution analysis was carried out with a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Instruments Ltd, UK). Measurements were done for Ti6Al4V particles dispersed in PBS, in PBS solution of human plasma fibrinogen and PBS solution of human plasma albumin (800 $\mu g \cdot ml^{-1}$). The granulometric curves obtained are shown on Fig 2. Particle size ranges from 3 µm to 70 µm, being the most probable value around 30 µm. Powder with adsorbed proteins aggregates in a certain small amount given place to aggregates of an average diameter of 350 µm.

2.1.1. Substrate hydrophilization

To induce the hydrophilization of surfaces, samples were exposed to an UV-C source for 15 h. This period was sufficient to guarantee a complete hydrophilization of the Ti6Al4V-particles surface [1]. To this purpose, a couple of TUV TL-D 15 W SLV lamps, kindly provided by Philips (Philips Ibérica, SAU, Madrid, Spain), emitting predominantly at a wavelength of 257.7 nm were used for UV-C irradiation of the particles, widely spread on polypropylene plates transparent to UV light. The lamp glass has filtering to avoid the production of ozone, which is produced by wavelengths lower than 200 nm. Plates were positioned exactly at 10 cm from each lamp receiving an intensity of 2.5mWcm^{-2}, during fixed time intervals. The whole irradiation system was situated inside an opaque wood chamber to avoid interferences from room or day light and any damage to users.

Immediately after the exposure to UV-C radiation the adsorption experiments were run to obtain the corresponding adsorption isotherms. The hydrophilized samples were referenced as Ti6Al4V-UV.

2.2. Protein adsorption

Protein adsorption experiments were carried out by incubation of 0.006-0.012 g of Ti6Al4V powder at 37 °C for 1 and 12 h in fibrinogen (Fg, Human Plasma Fibrinogen, fraction I, type I, 97% clottable, Sigma-Aldrich, USA) and albumin (HSA, Human Plasma Albumin, 97% clottable, Sigma-Aldrich, USA) solutions prepared in phosphate buffered saline solution (PBS, 8.7 g L^{-1} K_2HPO_4, 6.8 g L^{-1} KH_2PO_4 and 8.76 g L^{-1}NaCl, pH 7.4) in concentration range from 0 to 1400 µg mL^{-1}. Adsorption was performed in polypropylene tubes (1.5 ml, Eppendorf, Germany) that were placed in a vertical rotary mixer at 15 rpm.

After incubation, the mixtures were centrifuged and the residual protein concentration in the supernatant was determined spectrophotometrically at 337nm (Cary Eclipse, Varian Inc. Pty. Ltd., Australia,) by excitation at 280 nm. To evaluate the suitability of these measurements, several adsorption tests were done with labelled proteins (Human Plasma Fibrinogen, Alexia Fluor 488, Molecular Probes Inc., USA) and measured at 520 nm. There were not found any remarkable difference in the measured adsorbed amount for the whole concentration range studied between labelled and non-labelled proteins, thus all reported results herein were obtained from non-labelled proteins.

2.2.1. Adsorption isotherms

The amount of adsorbed proteins (Γ) was calculated using the relation (Eq.1):

$$\Gamma = [(C_o - C_e) \cdot V]/(m \cdot A_{sp}) \quad (1)$$

Where C_o is the protein concentration before adsorption, C_e is the protein concentration measured in the supernatant after the adsorption, V is the solution volume, m is the amount of Ti6Al4V exposed to the protein solutions and A_{sp} is the specific surface area of the Ti6Al4V powder.

Correction was made for protein adsorption to tube surfaces, pipettes, etc. by parallel incubation and handling of protein solutions. All determinations were carried out in triplicate, at least.

2.2.2. Zeta potential measurements

Zeta potential (ζ) measurements were carried out using an electrokinetic analyzer (EKA, Anton Paar KG, Graz, Austria) and analyzed as described previously for metallic samples[26]. Samples (1 g approximately) were introduced on a cylindrical screw powder cell (Powder Cell, Anton Paar KG, Graz, Austria) to keep the sample compacted between two nylon, 0.45 µm, microporous filter membranes (Merck Millipore, Germany). We checked that nylon membranes do not affect to the zeta potential measurements onto different control materials. This cell was inserted on a higher one (Cylindrical Cell, Anton Paar KG, Graz, Austria) which allows a close contact with the electrodes at both flanks and provide the electrolyte flow. The device was operated in an alternating pressure ramp form, applying a maximum pressure of 600 mbar. Each measurement was the average of 4 cycles. The external pH and conductivity sensors were always placed in the corresponding PBS reservoir. All measurements were carried out at least three times.

3. Results and discussion

A previous set of experiments were done to test the time needed to ensure the adsorption equilibrium. Despite the accommodation of proteins to the surface takes some time, it is only needed within 10-30 min, depending on the protein and the substrate, to obtain a film with a stationary concentration adsorbed [27-31]. However, to ensure conditions of equilibrium, adsorption experiments were conducted for one and twelve hours of contact. Within the experimental uncertainty, both isotherms were coincident for both proteins (data no shown). Therefore, one hour of contact between the powder and the chosen protein solutions was assumed as enough to ensure that the adsorption equilibrium was fulfilled.

Fig. 3 shows the adsorption isotherm of Fg on Ti6Al4V powder after 1 h of incubation time. The isotherm shows clearly two different behaviours. At the lowest C_e values, up to a coverage valued of 6 mg m^{-2} , the isotherm has a high slope, it suggests a strong adsorbent-protein interaction at the initial stages of the process. However, at the highest equilibrium concentrations, isotherm tends to a constant value of the retained amount. Transition from one to other section takes place through an elbow centered at the equilibrium concentration of 150 mg ml^{-1}. This behaviour is close to the reported adsorption onto titanium dioxide by *F. Höök et al.*[27]and *M. Pegueroles et al.*[23]. Also, accordingly to its shape, the isotherm can be fitted into the L2 type of the Giles´s classification, which is associated to a moderate adsorbent-adsorbate affinity, aimed the formation of an adsorbed monolayer.

Despite the limited application of the Langmuir model to our systems, experimental data fit adequately to Langmuir equation (Eq. 2) (r^2=0.89), where gamma, Γ_L, is the surface coverage at an equilibrium concentration, C_e, and a, b and c are fitting parameters related to the adsorption energy.

$$\Gamma_L = (a \cdot b \cdot C_e^{1-c})/(1 + b \cdot C_e^{1-c}) \quad (2)$$

The values obtained in our system were b = 0.022 mg ml^{-1} and Γ_L = 11 mg m^{-2} as monolayer capacity, or, taking into account the molecular weight of Fg, $1.84 \cdot 10^{-2}$ molecules nm^{-2}, which corresponds to an occupied area per molecule (A_L) of 55 nm^2 $molecule^{-1}$.

Fg molecule is not symmetric nor homogeneous, but a very anisotropic molecule (length/width ratio greater than 10), so that depending on its orientation, its interaction with the alloy surface can take place through different domains of the molecule. Also, because of its anisotropy the projected exclusion area on the adsorbent surface is different depending on its orientation. Assuming a minimum separation between molecules of 0.5 nm[31] and from the protein dimensions, the projected area is given by 48 nm x 7 nm, i.e. 336 nm^2, or 7 nm x 7 nm, i.e. 49 nm^2, for a flat or a perpendicular orientation on the surface, respectively. Consequently, the monolayer value obtained, 55 nm^2 $molecule^{-1}$, suggests a vertically oriented molecular layer on the surface (49 nm^2/molecule), probably with a certain degree of inclination to the surface or even hydrated.

Electrostatic interactions are important for protein adsorption to a given surface and the change of zeta potential with the evolution of the adsorption process can give valuable information about the progress of the adsorption[13,14]. The measured zeta potential values of the protein-covered surface were plotted in Fig. 3 as open squares (right axis) against the amount adsorbed of Fg. The zeta potential value of pristine Ti6Al4V powder was -13.75 ± 0.76 mV. Taking into account that zeta potential was measured at pH 7.4 and that the isoelectric point (IEP) of Fg is 5.5[32] both Fg and Ti6Al4V are negatively charged, therefore repulsion might be expected. However, Fg reorientation may overcome the repulsion, allowing adsorption by different functional groups looking at its electrical distribution. Adsorption progress reduces the electric absolute charge of the Ti6Al4V particles up to an approximated value of -6 mV, just from the early stages, at the lowest equilibrium concentration tested. Following progression in the adsorption, it is shown that the higher the concentration exposed, the lower the charge of the sample until a stable value of -4.0 mV, approximately. That value, within the experimental errors, is reached and maintained from the equilibrium concentration at which the plateau begins (400 µg ml^{-1}), so once the monolayer is formed, the zeta potential remains constant.

Fig. 4 shows the adsorption isotherm for Fg on hydrophilized Ti6Al4V powder. The analysis of the adsorption results indicates some interesting features. First, the shape of the isotherm of the hydrophilized alloy differs from that obtained for non hydrophilized sample. The initial slope of the isotherm is slightly lower than for the non-irradiated, i.e. the substrate hydrophilization causes a decrease on the affinity substrate-protein compared to that observed in the hydrophobic alloy, so that the new isotherm does not fit into the L2 subtype at Giles´s classification. From equilibrium concentrations around 70 mg ml^{-1} the isotherm adopts a convex shape, indicating the presence of a barrier in the adsorption process. In our previous study on the modification of the hydrophobicity by UV irradiation of Ti6Al4V [1], it was found the presence of more strongly adsorbed water on hydrophilic UV treated surface than on the non-treated Ti6Al4V. It could be expected that this water layer on the alloy surface may represent a barrier for the protein adsorption. However, this barrier is overcome with the progression of the adsorption, probably by cooperation with previously adsorbed molecules, as it is suggested for the high slope in this range of the adsorption isotherm; afterward the system reaches the same coverage than the hydrophobic surface.

It is also worth to mention that the retained amounts at higher equilibrium concentrations for both hydrophobic and hydrophilic surfaces are similar, i.e. the surface saturation is reached in both cases under the same conditions, with the surface coated by molecules mainly vertically oriented, regardless of the surface hydrophobicity. Therefore, this result indicates that from a certain concentration of ca. 500 mg ml^{-1}, the accumulation of proteins in both situations drives to a similar adsorption behaviour, guided almost exclusively by protein-protein interactions and independent on substrate hydrophobicity.

Comparisons of the measured zeta potential between hydrophobic and hydrophilic samples confirm the obtained results from the adsorption isotherm. There are no differences between irradiated and non-irradiated samples from the equilibrium concentration of 400 µg ml^{-1} in advance, However, the analysis that points to as the mayor presence of water at the interface, the lower the amount of adsorbed proteins, is consistent with the slightly lower zeta potential values obtained in this range for the irradiated alloy.

Fig. 5 shows the adsorption isotherm of HSA on Ti6Al4V powder. The slope in the concentration range 0 – 50 µg $ml^{-1}$of the HSA isotherm, $0.10 \cdot 10^{-3}$ m, seems to be relatively slightly lower than the obtained for Fg , $0.14 \cdot 10^{-3}$ m. However, the highest adsorption coverage (in mg m^{-2}) of HSA is nearly four times the obtained for Fg, which may be due to a more effective packing of the molecules of HSA because of its smaller size as compared with Fg.

Dimensions of HSA, with a molecular mass of 67 kDa[11], are 8 nm in length and 3 nm in width and in depth[33], so it can be modeled as rectangular equilateral triangle[34]. Admitting as for Fg a minimum separation between molecules of 0.5 nm, an exclusion zone of 32 nm^2, 30 nm^2 or 12 nm^2can be associated to its projected area depending on which triangle surface is exposed to the solid: the equilateral triangle area (8.5 x ($\sqrt{3}/2$) x 8.5 nm^2), the rectangular area defined between the equilateral triangles (8.5 x 3.5 nm^2) or simply the end corresponding to the contact of two rectangular surfaces assuming a certain degree of deformation to support the joint (3.5 x 3.5 nm^2).

Shape of the isotherm of HSA on Ti6Al4V suggests that adsorption takes place according two different mechanisms depending on the concentration range. Isotherm has a nearly vertical behavior (see inset in Fig. 5) up to a concentration of about 75 µg ml^{-1} where a small step appears. This tendency can be interpreted as the result of a very favorable protein-surface interaction, leading to an occupied area per molecule of 15 nm^2 $molecule^{-1}$. This value may correspond with the formation of a bilayer of HSA molecules, which would be consistent with the high trend of this protein to form dimers[35], each of them projecting an occupied area on the solid of 30 nm^2.

After that point, the adsorption rate slightly decreases up to a concentration of 180 µg ml^{-1} where a small plateau appears, corresponding to an area per molecule of 7 nm^2 $molecule^{-1}$. Adsorption progresses from this stage with a change in the tendency, as it were taken place over the previously adsorbed layer. This process continues up to a second plateau at 50 mg m^{-2}, which corresponds with an increase of almost 35 mg m^{-2} on the retained amount over the assumed dimer layer. The highest value corresponds to an occupation of ca. 2.5 nm^2 $molecule^{-1}$, which is compatible with a double bilayer of HSA dimers on the surface. These values are higher than those obtained by *F. Höök et al*.[27], *M. Pegueroles et al.* [23] and *F.Y. Olivia et al.*[36] on titanium dioxide, so HSA adsorbs thought a more efficient arrangement on Ti6Al4V than on TiO_2.

The zeta potential values of the protein-covered surface were plotted against the adsorbed amount of HSA on Fig. 5 together with the adsorption isotherm. For HSA, whose isoelectric point (IEP) is settled at 4.7[37], repulsion forces between protein and Ti6Al4V surface are present. As for Fg, the isotherm suggested that these forces are overcome by the orientation of the protein on the surface to its more favorable disposition.

Identically as Fg, the zeta potential rises toward ca. -7.0 mV when the first HSA monolayer of dimers is completed, being nearly constant afterward. This value is slightly more negative than the obtained for Fg (-4.0 mV). These could be a consequence of the difference between the IEP of both proteins may be supported because HSA is more negative than Fg at pH 7.4.

Fig. 6 shows the adsorption isotherm for HSA on Ti6Al4V powder previously hydrophilized by with UV. Except at the lowest coverages (see inset in Fig. 6), the adsorbed amount at any given equilibrium concentration is lower for the hydrophilic condition. Up to coverages of ca. 5 mg m^{-2} both isotherms are coincident. This value corresponds to the beginning of the region where adsorption seems to progress by dimeric arrangements on the surface in the isotherm of the hydrophobic Ti6Al4V. This suggests that the high hydrophilicity of the substrate hampers the stable arrangements of dimers, which were favored by the hydrophobic interactions with the substrate of the former adsorbed molecule of the dimer. It should be noted that the whole isotherm for the hydrophilic surface is substantially continuous, with no clearly singular behaviour. This fact suggests, beside the absence of the possible dimeric rearrangement at the hydrophobic surface, that adsorption on the hydrophilic Ti6Al4V surface seems to progress continuously but with a worse packing of the HSA molecules, that can justify the lower coverages attained in the hydrophilic solid as compared with the hydrophobic.

Analysis of the zeta potential values reveals only slightly differences located at the lower protein concentrations between the hydrophobic and hydrophilic samples, identically as for Fg. Furthermore it is also worth mention that HSA produce a more gradually neutralization of the Ti6Al4V hydrophilized surface than Fg. As can be observed, the initial slope of these zeta potential values is not as pronounced as for the corresponding for Fg adsorption. This difference could be due to a slightly more negative nature of HAS, as justified before and, probably more important, to it lower size which allows better arrangements on the surface.

4. Conclusions

It has been explored the feasibility of powdered Ti6Al4V as a material to produce biomaterials such as scaffolds. Both Human Fibrinogen and Albumin absorption isotherms have been obtained accompanied with an electric characterization of the formed systems.

Despite the fact that both proteins are able to overcome the electrostatic repulsion with the powder surface, fibrinogen seems to form a saturated interface with molecules preferably vertically oriented, while albumin adsorption moves sequentially over the surface of the Ti6Al4V particles trough dimmer formation, progressing adsorption over this initial bilayer.

On the other hand, Ti6Al4V powder hydrophilization has been revealed as a hinder parameter for both proteins adsorption but in a different manner. The fibrinogen saturation degree is limited at

early absorption stages while this limitation affects the whole range studied for albumin. Zeta-potential values are in agreement with both facts.

The possibility of using powdered Ti6Al4V as adsorbent has allowed getting a deeper and detailed knowledge on the interaction of Fg and HSA with both hydrophobic and hydrophilic alloy, together with a valuable information about powdered material for its use in prosthesis fabrication. Despite both proteins are able to overcome the electrostatic repulsion with the powder surface, fibrinogen is adsorbed on the Ti6Al4V powder forming a saturated interface with molecules preferably vertically oriented, while albumin adsorption moves sequentially over the surface of the Ti6Al4V particles trough dimmer formation, progressing adsorption over this initial bilayer.

On the other hand, Ti6Al4V powder hydrophilization hinders both proteins adsorption. In the case of Fg, the saturation degree of the surface is not altered respect with the hydrophobic surface, but for albumin limits the total amount retained. Zeta-potential values are in agreement with both facts.

Acknowledgments

JRS would like to thank to the Ministerio de Educación, Cultura y Deporte (Government of Spain) for his grant FPU2013/00862. Also authors would like to thanks to project MAT2009-14695-C04-01 Ministerio de Ciencia e Innovación, Grant GR10149 of Consejería de Economía, Ciencia e Innovación (Junta de Extremadura), FEDER funds, NANBIOSIS ICTS, more specifically to the Surface Characterization Unit of the Biomedical Networking Center (CIBER-BBN) and the SACSS-SAIUEx of the University of Extremadura (UEx) for technical support. We thanks to IESMAT for Laser Diffraction measurements of particle size.

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Figure Captions

Fig. 1. Scanning electron micrograph of Ti6Al4V particles.

Fig. 2. Particle size distribution curves for water dispersions of i) Ti6Al4V particles, ii) Ti6Al4V particles in the presence of HSA and iii) Ti6Al4V particles in the presence of Fg obtained by laser diffraction.

Fig. 3. Adsorption isotherm of Fg on Ti6Al4V powder (black circles) in PBS after 1 h of incubation time and its ζ potential (open squares) dependence with the equilibrium concentration C_e. The patterned area represents the average error for the isotherm.

Fig. 4. Adsorption isotherm of Fg on Ti6Al4V powder previously hydrophilized by UV-C irradiation (black circles) in PBS after 1 h of incubation time and its ζ potential (open squares) dependence with the equilibrium concentration C_e. The patterned area represents the average error for the isotherm.

Fig. 5. Adsorption isotherm of HSA on Ti6Al4V powder (black circles) in PBS after 1 h of incubation time and its ζ potential (open squares) dependence with the equilibrium concentration C_e. The patterned area represents the average error for the isotherm. The inset show magnificated region to facilitate the appreciation of the progressively adsorption process.

Fig. 6. Adsorption isotherm of HSA on Ti6Al4V powder previously hydrophilized by UV-C irradiation (black circles) in PBS after 1 h of incubation time and its ζ potential (open squares) dependence with the equilibrium concentration C_e. The patterned area represents the average error for the isotherm. The inset show magnificated region to facilitate the appreciation of the progressively adsorption process.

Table 1. Percentage composition of the samples surface from XPS. Residual traces of other elements at a rate < 2% are not included. Relative values of percentages between Ti2p, Al2p and V2p3 are included.