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Experimental approach towards the water contact angle value on the biomaterial alloy Ti6Al4V

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In the biomedical field, water contact angle is a useful gauge to follow how a biomaterial surface would interact with the surrounding water-like physiological environment. Ti6Al4V alloy is widely used in orthopedic applications. Nevertheless, the values of its water contact angle reported in the literature show a large dispersion, from 40° up 80°. However, in addition to the expected dependence of the surface wettability on preliminary treatments, the values of the water contact angle on the pristine Ti6Al4V alloy suffers from an important variability and lack of reproducibility. The present research pays attention to this difficulty and proposes a simple experimental procedure to ensure adequate contact angle reproducibility. Controlled passivation growth in mild underwater conditions of freshly polished disks, followed by ultrasonic washing, avoiding the rubbing of the surface, gives average water contact angles of 80° with very low standard deviations also among samples from the same batch.

1. INTRODUCTION

Analysis of the shape of liquid drops on a solid is one of the most powerful methods for surface characterization. The contact angle formed by a liquid drop on a solid is dependent on the properties of the uppermost molecular layers of the solid, consequently the presence of any coverage or adsorbed layer on the solid affects the shape of the settled drops on the solid. For this reason, measurements of contact angles of probe liquids on solids are successfully used to characterize surface treatments or adsorbed films on solids [1-8].

Among the titanium-based alloys, Ti6Al4V is widely used in orthopedic applications. It is an *α/β* titanium alloy, each phase being stabilized with aluminum and vanadium, respectively. Microstructure of the alloy is related to the processing conditions, as temperature, annealing time or cooling rate, together with the distribution of alpha or beta phases. Once exposed to atmosphere a passive layer is formed on the surface of Ti6Al4V almost instantaneously. This layer is mainly composed by titanium dioxide, even though other oxides of titanium and of the alloying elements are also present.

The success of Ti6Al4V in the biomedical field comes from its good biocompatibility and mechanical and anti-corrosive properties [9]. Interactions between an implant and the host tissue are mediated by the outermost layers of the implant. Therefore most of the reasons of the biocompatibility of any material are related to its surface characteristics even more than with its bulk properties. Besides, in spite of the suitable behavior of this alloy, nowadays there are considerable attempts to improve its properties by dressing the surface with active coverages. Efforts are under way to provide the surface with molecules that could help in given pathologies, such as osteoporosis, or to prevent infections by avoiding bacterial adhesion. However, it is essential for the design and reproducibility of any functionalization process to have thorough knowledge of the physicochemical properties of the pristine surface, as its wettability and surface free energy. For this reason, the surface characterization of the biomaterial is crucial.

There are several studies dealing with the effects of sterilization on the surface properties of Titanium implants [10-12]. Among them J.H. Park et al. [12] studied implants of unalloyed Titanium and showed that depending on the cleaning methodology and sterilization technique, as autoclaving, gamma irradiation, oxygen plasma or UV illumination, surface properties of Titanium, such as wettability are modified and the

biomaterial biological response are altered. These authors concluded that a second sterilization may not give the same responses than unused samples.

Ti6Al4V alloy is expected to behave similarly to Titanium. Bibliography shows water contact angle values ranging from about 80º to 40º [13, 14]. In addition, it is difficult to understand how a specific surface treatment changes the wettability of Ti6Al4 since different authors provide different information for the same surface modification. For example, D. E. MacDonald et al. [15] characterized thermally and chemically modified Ti6Al4V alloy disks by contact angle measurements, finding a difference of more than thirty degrees between the control sample and the thermally modified samples. On the other hand, R. A. Gittens et al. [16] observed that the heat oxidation treatment did not produce a large variation in surface wettability.

Moreover, an important practical problem related to the evaluation of the contact angles on this alloy has been observed. Wettability determination used to be done by measurements of contact angle of settled water drops on polished disks of the alloy. These disks come generally from cutting off hot rolled and annealed bars of Ti6Al4V ELI. These bars are two or three meters long and are available with different diameters, to attend to the needs of implant manufacturers. Nevertheless, despite tests were done with disks from the same batch, dispersion on the measured values of water contact angle is much larger than could be expected on highly polished materials. In a previous study on the effect of UV radiation on the wettability of disks of Ti6Al4V we found a radial dependence that was correlated with microstructural differences within the bar [17]. The present research has been based on the hypothesis that the variability in water contact angles for Ti6Al4V can be due to the heterogeneity of the samples surfaces caused by singularities in the bulk properties of the material and to the variations in the cleaning and measuring protocols.

On this basis, the objective of this work is to analyze the water contact angle of Ti6Al4V samples by considering different cleaning and measuring protocols and looking for a treatment of samples that could ensure an adequate contact angle reproducibility, which is a basic condition for adequate further functionalizations of this alloy.

2. MATERIALS AND METHODS

Commercially available Ti6Al4V (DKSH, Switzerland) was used. Discs of 25 mm in diameter and 2 mm in thickness were cut from a single bar. The samples were mechanically polished to mirror finish and carefully cleaned before used. To obtain a mirror finish, disks were successively abraded using silicon carbide papers and polished with diamond paste, finishing with silica colloidal (all polishing materials were supplied by Buehler, Germany).

Once polished, the samples were cleaned following the protocols listed below by using different compounds: antiseptic liquid (Derquim DSF, Panreac, Spain), distilled water, acetone (Panreac, Spain) and ethanol (Panreac, Spain).

After cleaning, some disks were dried with a nitrogen flow. Other disks were dried for one hour in an oven at 120°C, protected with a cover to avoid any dust deposition from the oven; afterward, samples were deposited in a desiccator for equilibrating their temperature.

Water contact angles on disks were measured using the sessile drop technique with a G20 goniometer (Krüss, Germany). The ultrapure water (Milli-Q Plus) droplet volume (5 mm^3) was precisely controlled with an automatic syringe. To measure the contact angle, samples were placed in a thermostatic chamber filled with water at a constant temperature of 25°C for 20 min.

Protocol #0:

Samples were cleaned by rubbing the freshly polished surfaces with a cotton strip impregnated with the antiseptic liquid. Then disks were ultrasonically cleaned for 10 min in successive baths of distilled water, acetone and ethanol. Finally samples were dried under N_2 flow and deposited in a desiccator for 60 min.

Protocol #1:

Samples were cleaned avoiding rubbing. Disks were ultrasonically cleaned for 10 min in successive baths of the antiseptic liquid, distilled water, acetone and ethanol. Then, samples were allowed to dry under two different conditions: some disks were dried with an N_2 stream and others with the help of an oven at 120°C. Afterward, samples were deposited in a desiccator for two different periods of time: 60 and 120 min. Four samples were used for each drying-conditions (two for each desiccator-time). Three drops were deposited for each sample following the diagonal line.

After the first measurement, drops were removed with absorbent paper and samples were again cleaned and dried as described before. Afterward the measurements of water contact angles were repeated.

Protocol #2:

Eight samples, denoted as A, B, C, D, E, F, G, H, were taken in this case. Samples were not randomly studied; instead, the contact angle analyses were done separately for each one. The cleaning protocol was the same as Protocol #1. The drying was carried out under N_2 flow and dessicator-time was fixed to 60 min. Seven drops were deposited across the entire surface of each sample.

Protocol #3:

A new group of samples were used to homogenize the passive layer on the Ti6Al4V surface. After cleaning ultrasonically for 10 min in the antiseptic liquid, water, acetone and ethanol, samples were introduced into a container of Milli-Q water for 24 h, 48h, and 72 h in the oven at 50°C. After that time, samples were removed from the container, dried in the oven for 1 h at 120°C and deposited in a desiccator to equilibrate their temperature for 60 min. Some of the samples introduced in water for 72 h were also maintained inside the desiccator for 24 h to check if temperature-equilibration time affects the results. Contact angles were measured by depositing seven drops of water on the entire surface of each sample.

The results will be presented in box-whisker plots. The median is shown by a line dividing the box into two parts. The average is marked as a point inside the box. Lower whisker marks the score of 5th percentile and upper whiskers refers to the score of 95th percentile.

3. RESULTS AND DISCUSSION

Figs 1-3 show the results obtained for samples differently prepared prior to the measurements. The cleaning procedures have been based on previous bibliographic information and our own experience [14, 18, 19]. The first set of experiments was done by cleaning the samples as described in Protocol #0. After different steps of cleaning by rubbing the samples with a cotton strip the values of water contact angles showed a high dispersion (between 46° and 73°) and the results depended on the sample selected. Lilley et al. have pointed out that the adherence of the passive film to the pristine surface of Titanium is rather poor, and it may

be disrupted at very low shear stresses, even rubbing against soft tissues [19]. Since the samples were cut from the same Ti6Al4V bar, the reason for the variability in the contact angle observed in the case of samples from Protocol #0 could be related to alterations caused by the rubbing process in the passive film spontaneously formed on the alloy samples. Therefore, the further cleaning protocols avoided the rubbing of samples.

Fig. 1 presents the distribution of data following the cleaning Protocol #1. In this case, the Ti6Al4V samples were randomly selected, cleaned as described before and dried under two different conditions: with a nitrogen flow or in an oven (referred to as N_2 and Oven in abscissa, respectively).
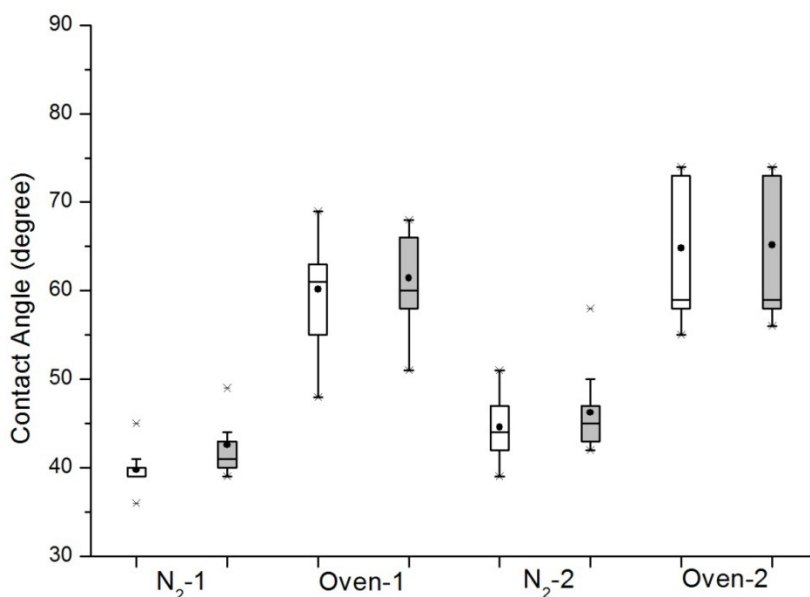

Fig. 1. Water contact angles measured on samples cleaned following the Protocol #1. Samples were dried with nitrogen flow (N_2) or in an oven. 1 is referred to data after the first washing process and 2 is referred to data after a second washing process. White and grey boxes present the results of 60 min and 120 min of temperature-equilibration time, respectively.

After drying, samples were maintained in a desiccator for 60 or 120 min (white and grey boxes, respectively). In both cases, samples were examined a second time after following the same cleaning protocol. There are two important observations: the higher values of contact angles for oven-dried samples compared to N_2-dried samples and the higher dispersion in the results distribution for oven-dried samples. Average

values of contact angles for oven-dried samples were between 60° and 65° while for N_2-dried samples were between 40° and 45°. In oven-dried disks, the surface acquires a contamination layer during the drying process and the hydrophobicity increases. When samples are cleaned for a second time the data dispersion seems to increase and average values are slightly higher. In relation to the desiccator-time there is no clear effect on the contact angle, data distribution after 60 min and after 120 min seems very similar, no matter which drying procedure was followed (N_2 or oven). At this point it is worth mentioning that some experiments were made after keeping the samples for 3 days in the dessicator and the obtained results were similar to those presented for 60 or 120 min.

In order to check whether the observed variability was due to possible different characteristics between samples we made experiments following the same cleaning protocol but labeling each one of the samples. As previously described in Protocol #2, eight samples, denoted as A, B, C, D, E, F, G, H, were taken. Each sample was also cleaned twice and its contact angles measured after the first and the second wash. Desiccator time was fixed at 60 min. Results are presented in Fig. 2.
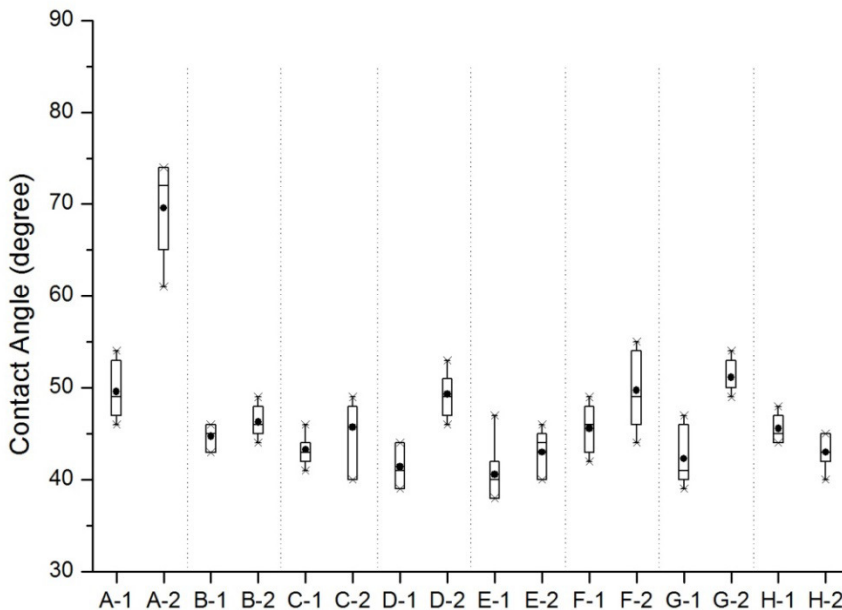

Fig. 2. Water contact angles measured on samples cleaned following the Protocol #2. Letters A – H represents each one of the different eight samples analyzed. 1 is referred to data after the first washing process and 2 is referred to data after a second washing process.

It is observed again, regardless of the selected sample, that, in general, the second wash always increases the average contact angles. After the first cleaning process contact angles were comprised between 38° and 48° in samples from B to H, but the A sample gets contact angles ranging from 47° to 54°. This reflects the different behavior of the samples and so, standard deviations increases when averaging data from all the samples. However, focusing in each individual sample, standard deviations were, in general, low, within 1° – 3°. After the second cleaning process the contact angle for samples B - H was within 40° – 55° and for sample A within 61° – 74° with standard deviations between 2° – 5°, higher than after the first washing step. Although this last measurement protocol is able to reduce standard deviations for samples washed once, the specific values of water contact angles depends on the sample, i.e. the specific wettability depends on the disk analyzed. However, wettability should reflect the standard behaviour of the biomaterial but it should not be referred to single samples.

Among the reasons behind the observed diversity in water contact angles it could be liable the passivation layer spontaneously formed on the Ti6Al4V surface. The passivation process begins just once the pristine surface contacts with oxygen, but the passive layer grows slowly with time, reaching a thickness of about 6 nm after one year exposure to normal ambient temperature [20]. This dynamic oxidation process results in differences in the passivation layers of samples exposed to the same ambient conditions for different periods of time and, consequently, their wettability can be also different. Moreover the singularities in the bulk properties of the material can be somewhat translated to the properties exhibited by the passivation layer. For example, Faghhi et al. observed that osteoblast adhesion was improved on fine grained in relation to regular grained titanium, suggesting that the most external layer of the material could be affected by the properties of the bulk [21]. A similar behavior was also found for Ti6Al4V [22]. The influence of van der Waals interactions from the bulk material to the top surface can be also transferred to the contact angle information, since van der Waals forces range over tens of nanometers [23]. Hähl et al. [24] studied the adsorbed layer of globular proteins on silicon wafers with silicon oxide layers of different thickness. They proved that the characteristics of the adsorbed layers of proteins were affected not only by a few top layers of atoms on the surface, but also were sensitive to the van der Waals forces from the subsurface layers.

On these bases, it would merit to obtain a more homogenous passivation layer on the Ti6Al4V surface in order to minimize the possible contribution from the bulk characteristics to the outer surface and to avoid deviations associated with the irregular layer thickness that is formed spontaneously. Thus, as described in detail in Protocol #3, the passivation layer was grown enough in underwater conditions for three different periods of time, 24 h, 48 h and 72 h (passivation-growth time), setting a mild temperature of 50°C inside an oven, to avoid any possible activation of ions diffusion or any other thermal process. The results of these experiments are summarized in Fig. 3.
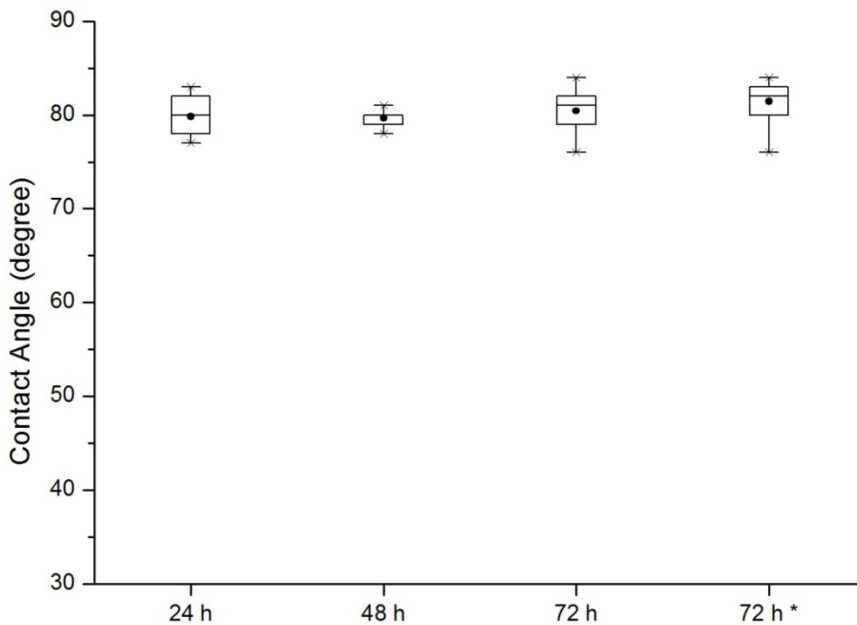

Fig. 3. Water contact angles measured on samples cleaned following the Protocol #3. 24h, 48h and 72h are referred to the different times that the samples was maintained underwater at 50°C to promote the growth of the passive layer. The first three boxes belong to samples kept inside the desiccator for 60 min and the last one was kept for 24 h (*).

This figure clearly shows that a stable value of the average contact angle was obtained, regardless of the passivation-growth-time. Also, the data distribution is very similar in all cases, with the lowest dispersion observed for measurements made at 48 h. It is also interesting to point out that the average value of contact angle is about 80°, higher than any of those obtained with other preparation protocols. It is likely that this

procedure allows a thicker and more uniform passive layer of Ti6Al4V and therefore a lower dispersion in contact angle. The decrease in the observed wettability can also inform about the poor contribution of the bulk material properties to this passivation layer and it must be related to the protecting character of such hydrophobic passivation layer in water-like environments [25-27].

4. CONCLUSIONS

The wettability of Ti6Al4V surfaces is highly dependent on their past history. In particular, the thickness of the passivation layer and the surface cleaning protocols are crucial in the quantification of its hydrophobicity through water contact angle measurements.

Controlled passivation growth in underwater conditions of freshly polished disks, followed by an ultrasonic washing process, employing different cleaning compounds (antiseptic liquid, acetone and ethanol) and always avoiding the rubbing of the surface, gives average water contact angles of 80° with very low standard deviations.

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