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A New View of a:C-H-coated Ti-6Al-4V Alloy to be Used as Orthopedic Implants: Influence of Surface Free-energy of Interaction on the Biological Responses

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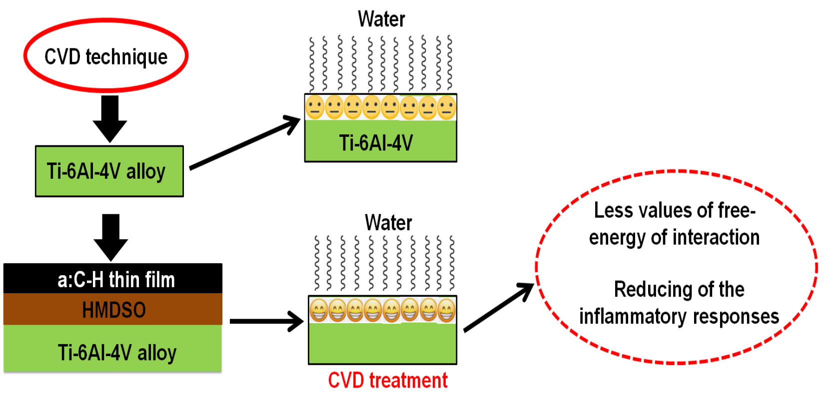
In a previous published work, the Ti-6Al-4V alloy were functionalized by using plasma enhanced chemical vapour deposition (PECVD) process in order to obtain hydrogenated amorphous carbon (a:C-H) on the biomaterial's surface. At the time, we noticed the functionalized surface contributed to the advance of using Ti-6Al-4V in biomedical implants applications as well as in the adding of intrinsic properties as demonstrated by electrochemical and biological assays. Here, we present new view of a-C:H-coated Ti-6Al-4V alloy, considering the influence of surface free-energy of interaction and understanding how the surface influences the biological response using pre-osteoblastic cells (MC3T3-61). Our results demonstrate, the Ti-6Al-4V containing a:C-H film has less negative value of surface free-energy when compared to the bare material (-22.156 mJ m^{-2} *vs.* -60.046 mJ m^{-2}), suggesting a tendency toward to a less hydrophobic surface which is extremely important to reduce the inflammatory process, common in orthopedic implants.

Graphical abstract



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1. Introduction

Titanium alloys (Ti-alloys) have been widely used commercially and industrially due to their diverse applications, such as: airplanes, naval ships, missiles, spacecraft and biomedical sector (dental and orthopedic) [1, 2]. Ti-alloys

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display good mechanical and corrosive resistance, low density, resistance to high temperatures and biocompatibility [3, 4]. Among the different combinations of Ti, the Ti-6Al-4V alloy is widely used in orthopaedic and dental applications for bone replacement.

Surgical implantation of artificial biomaterials is the most widespread solution to remedy musculoskeletal problems, being used in several parts of the human body: replacement of shoulders, knees, hips, elbows, orthodontic structures, among others. As reported by Eurídice et al [5], the success of these biomaterials depends on the response they induce in the body as well as the material's degradation in the body environment, which are directly related to their mechanical and corrosive properties. As is well known, TiO_2 thin films act as a natural protective barrier, however, when exposed to aggressive media the film ends up breaking and exposing the Ti-6Al-4V alloy to a global and localised corrosion processes, respectively. The migration of Al and V elements from the Ti-6Al-4V alloy to the human body can promote neurological diseases such as Alzheimer and Parkinson.

The search for alternatives that may minimize this action is extremely important for the application of the Ti-6Al-4V as biomaterial. In a previous published work [5], hydrogenated amorphous carbon (a-C:H) have been deposited onto substrate of Ti-6Al-4V alloy by PECVD technique using CH_4 gas as precursor in order to contribute with refined data on the promising its application in medical devices. We verified by using potentiodynamic polarization curves (PPc) and electrochemical impedance spectroscopy (EIS) tests that a-C:H film acts as a protective barrier against corrosion process in an artificial saliva media. In biocompatibility assays, when human peripheral blood mononuclear cells (PBMCs) were evaluated, only cells cultured on the alloy without a-C:H had significant induction of the apoptotic process.

Another extremely important consideration that must be taken into account to determine the biocompatibility of the material/host interface is related to the surface wettability. As reported by Kim et al [6], the surface composition and hydrophilicity are parameters that may play a role in implant-tissue interaction and osseointegration. In other words, the surface free energy of interaction is an important parameter that should be considered for more robust applications of biomaterials. In the present short communication, we report new results of the Ti-6Al-4V alloy containing a:C-H film, based on surface free energy of interaction. The cell viability was assessed using pre-osteoblastic cells (MC3T3-61).

2. Material and Methods

In a previous published work, a set of a:C-H films were obtained by PECVD technique at the Laboratory of Technology and Surface Engineering (LabTES) at the Sorocaba Technological College, Sorocaba, Brazil. Further details of the morphological, structural and electrochemical characterization of this system may be obtained in reference [5]. Here, we used the same procedure to obtain a:C-H thin films on the Ti-6Al-4V surfaces. For this purpose, the deposition process was performed at the Laboratory of Thin Films and Plasma Processes at the Federal University of Triângulo Mineiro (UFTM).

2.1. Wettability measurements

The contact angles (θ) between the surface and water (Mili-Q), α-bromonaphthalene (Merck) and formamide (LGC Bio, São Paulo, Brazil) were determined by using a goniometer (Krüss, Hamburg, Germany). The sessile drop method was used to measure the contact angle (θ) for all liquids and Ti-6Al-4V alloy uncoated and coated a:C-H film surfaces. The experiments were conducted in triplicate and the measurements were obtained for 30 s in a presence of each liquid used. Figure 1 (a) shows a schematic representation of a droplet on a solid surface. As can be seen, the forces exerted by the surface tensions are represented by the three arrows: solid-vapour (γ_{sv}), liquid-solid (γ_{ls}) and liquid-vapour (γ_{lv}). As shown in Figure 1 (b), the droplet on a solid surface, depending on the physical properties of the investigated material may exhibit different behaviors as a function of the contact angle. In this sense, depending of the hydrophobicity of the studied material, biological applications may be facilitated.

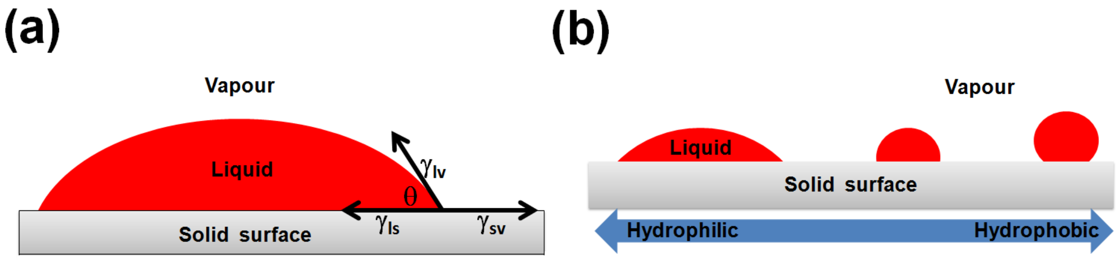

Fig. 1. Schematic representation of the static contact angle (θ) measurement by using sessile drop method (a) and a varying contact angle, showing the hydrophobicity of the material surface (b).

The Young–Dupré equation (see Equation 1) describes this balance.

$$\gamma_{sv} = \gamma_{lv} \cos\theta + \gamma_{ls} \quad (1)$$

and solving, the contact angle may be written as:

$$\cos\theta = \frac{\gamma_{sv} - \gamma_{ls}}{\gamma_{lv}} \quad (2)$$

when θ > 90°, there is no wetting of the solid by the liquid, i.e., there is no spreading of the liquid (hydrophobic surface). When θ < 90°, the wetting occurs and the liquid spreads spontaneously on the solid (hydrophilic surface). If the angle is greater than 165° or equal to 180°, the surface is called superhydrophobic and when θ ≈ 0°, the liquid spreads indefinitely on the solid.

The Van Oss approach [6] was used to determine the surface free energy of interaction of the Ti-6Al-4V uncoated and coated a-C:H films. The Van Oss approach relates the Lewis acid–base γ_i^{AB} and Lifshitz–van der Waals apolar component γ_i^{LW}. As known, these interactions may be represented by Equation 3:

$$\gamma_i = \gamma_i^{LW} + \gamma^{AB} = \gamma_i^{LW} + (\gamma_i^{+}\gamma_i^{-})^{1/2} \qquad (3)$$

where the γ_i^{LW} represents the Lifshitz-van der Walls (LW) component of surface free energy, γ^{AB} the polar acid-base component of surface free energy, γ_i^{-} the component of γ^{AB} describing the Lewis base and γ_i^{+} the component of γ^{AB} representing the Lewis acid. Considering a drop of liquid on a given solid surface and calculating the components as a function of the contact angle it is possible to obtain Equation 4.

$$\gamma_L^{Total}(1 + \cos\theta) = 2\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + 2\sqrt{\gamma_s^{-}\gamma_l^{+}} + 2\sqrt{\gamma_s^{+}\gamma_l^{-}} \qquad (4)$$

The total free energy of interaction (ΔG_{sws}^{Total}) among molecules of the surface immersed in water, formamide and α–bromonapththalene may be determined by the sum of the polar (ΔG_{sws}^{AB}) and nonpolar (ΔG_{sws}^{LW}) free energy of interaction according to Equation 5:

$$\Delta G_{sws}^{Total} = \Delta G_{sws}^{AB} + \Delta G_{sws}^{LW} \qquad (5)$$

2.2. Biological assays

Cytotoxicity tests were performed on the Ti-6Al-4V and Ti-6Al-4V alloy containing a:C-H film by using direct contact as described by ISO 10993-5 [7]. For this purpose, pre-osteoblastic cells (MC3T3-61), obtained from the American Type Culture Collection (ATCC), were cultured in minimum essential medium α (MEM α), supplemented with fetal bovine serum (FBS) 10%, 5 µg mL^{-1} of ascorbic acid, 7 mM of β-glycerophosphate, 10,000 U mL^{-1} of penicillin G and 10,000 µg mL^{-1} of streptomycin [8]. Cells were plated at a density of 4 x 10^5 cells per 6-well plates in α-MEM medium. The cell viability was determined after 24 h by flow cytometry in quintuplicate.

3. Results and Discussion

Cell viability was evaluated by means of the flow cytometry (FCM) analysis. As shown in Figure 2, the coated and uncoated material did not show cytotoxic effect to osteoblastic cells, demonstrating a cell viability of 97.3 ± 7.7% and 96.5 ± 7.3, respectively. The measurement of the contact angle formed by a droplet of liquid (water, formamide, α–bromonapththalene) placed on a horizontal surface indicates the coated material displays a tendency towards to acts as a hydrophilic surface (θ < 90°) (see Figure 3). As is known, the contact angles are closely related to the ΔG_{sws}^{Total}. Here, the values of polar, ΔG_{sws}^{AB}, and nonpolar compounds, ΔG_{sws}^{LW}, of the total free energy of interaction, ΔG_{sws}^{Total}, of the coated and uncoated materials are presented in Table 1.

Table 1. Values of polar (ΔG_{sws}^{AB}) and nonpolar compounds (ΔG_{sws}^{LW}) of the total free energy of interaction of uncoated and coated Ti-6Al-4V alloy.

Surfaces	ΔG_{sws}^{LW} **(mJ m^{-2})**	ΔG_{sws}^{AB} **(mJ m^{-2})**	ΔG_{sws}^{Total} **(mJ m^{-2})**
Ti-6Al-4V alloy	-3.127	-56. 919	-60.046
Ti-6Al-4V + a:C-H film	-3.176	-18.979	-22.156

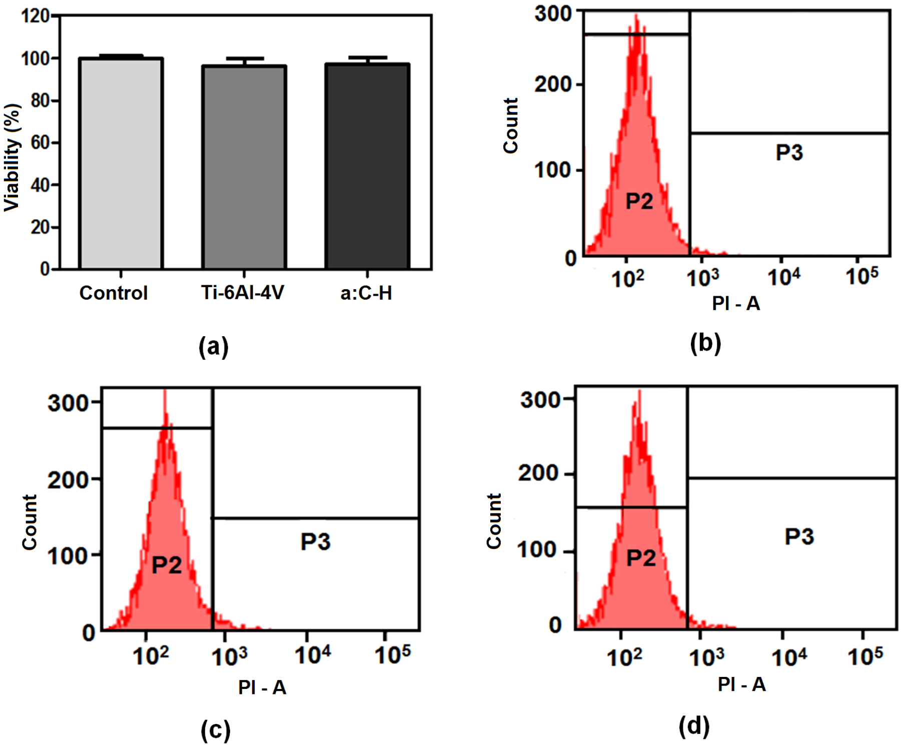

Fig. 2. Cytotoxicity assays by using FCM analysis (a) and (b, d) flow cytometry of osteoblastic cells. (b) Dot plot of positive control, (c) Dot plot of cells deposited on the Ti-6Al-4V surface and (d) Dot plot of cells deposited on the Ti-6Al-4V + a:C-H film. P2 represents viable cells and P3 non-viable cells. Results were expressed as mean ± standard deviation; * p <0.05 (One-way ANOVA followed by Tukey test) (n = 5).

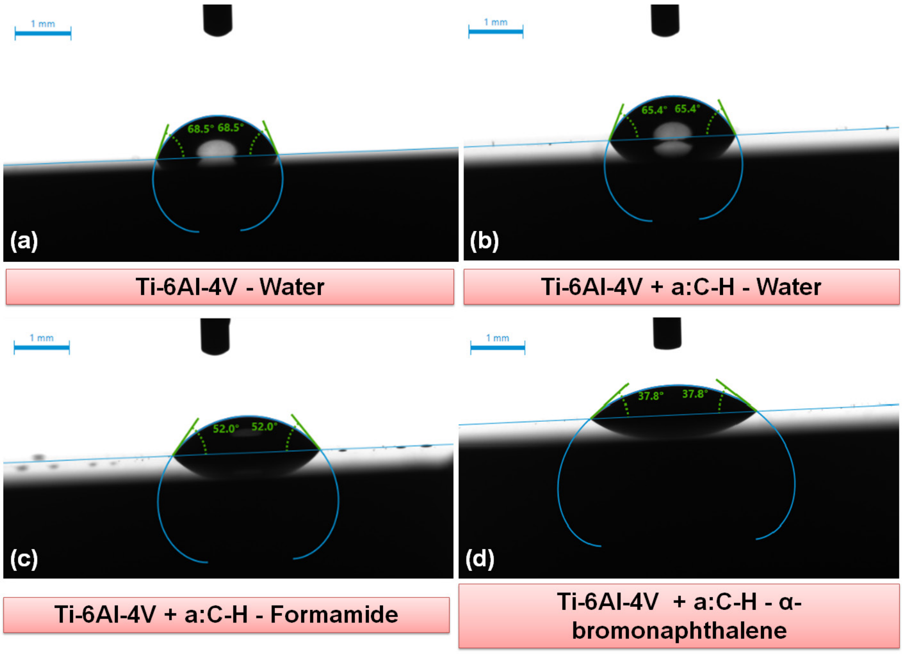

Fig. 3. Results of wettability measurements (a) Ti-6Al-4V in water, (b) Ti-6Al-4V + a:C-H in water, (c) Ti-6Al-4V + a:C-H in α–bromonapththalene and (d) Ti-6Al-4V + a:C-H in Formamide.

As reported by Araújo et al [8], value of $\Delta \boldsymbol{G}_{\mathbf{sws}}^{\mathbf{Total}}<0$ suggest the material surface has a tendency towards to be hydrophobic, i.e., when immersed in water the molecules of the surface interact with each other rather than forming an interface with water. Our results demonstrate, the Ti-6Al-4V containing a:C-H film has less negative value of $\Delta \boldsymbol{G}_{\mathbf{sws}}^{\mathbf{Total}}$ when compared to the bare material (-22.156 mJ m^{-2} *vs.* -60.046 mJ m^{-2}), suggesting a less hydrophobic surface. In fact, hydrophilicity is a good surface property for biomedical devices related to osseointegration, since a low inflammatory response is desired. According to Tang et al [9], cell adhesion and inflammatory response are directly related, among others, to hydrophilic surface properties. Hydrophilic surfaces provide a lower amount and less tightly proteins bind an inhibition of leukocyte adhesion as well. Consequently, a lower inflammatory response, with a reduction of pro-inflammatory cytokine levels is expected. These observations are in agreement with the results obtained in a previous published work [5], which we demonstrated the production of IL-2, IL-4 and IL-5 cytokines is mostly induced by CD4+ T lymphocytes, which in our study showed no induction of apoptosis or any sign of activation when the titanium alloy were coated with a:C-H film. Hotchkiss et al [10] report that hydrophilic titanium surface induced macrophage activation similar to the anti-inflammatory M2-like state. Also, surface hydrophilicity would improve the soft tissue integration of dental implants, irrespective of material composition [11]. In this sense, the results obtained in the present work are extremely interesting once we demonstrated the CVD method was beneficial for a:C-H films production on the Ti-6Al-4V alloy surfaces as well as in the reduction of inflammatory process. The influence of free energy of interaction on the biological assays were demonstrated, validating the importance of the surface hydrophobicity for different applications.

4. Conclusions

Here, we complement a previous published work, bringing new insights to the biomedical field. In addition, it was possible to validate the importance of the surface hydrophobicity as well as understanding the reduce of the inflammatory responses is linked to the Ti-6Al-4V containing a:C-H film, which presented less value of surface-free energy of interaction when compared to base material.

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Author Contributions

The authors' contributions are J.P.L do Nascimento – Investigation, writing - review & editing; L. R. Freitas – Investigation, writing - review & editing; C. N. Lemos – Biological assays and formal analysis; R.V. Gelamo – Deposition process and formal analysis; J.A. Moreto – Project administration, Supervision, Formal analysis.

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