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The influence of surface energy on competitive protein adsorption on oxidized NiTi surfaces

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Abstract

NiTi shape memory alloy surfaces, untreated, and oxidized by a new oxidation treatment (OT) in order to obtain a Ni-free surface, have been compared in terms of surface energy and protein adsorption behavior. The polar and dispersive components of the surface energy have been determined. A competitive adsorption process between fibronectin and albumin has been carried out by ^{125}I-radiolabeling. Moreover, the adhesion strength between both proteins and NiTi surfaces has been evaluated by performing an elution test. The results show that the OT treatment enhances the hydrophilic character of NiTi surfaces by significantly increasing the polar component of their surface energy. Moreover, the OT treatment increases the amount of fibronectin and albumin adsorbed. It also increases the fibronectin affinity for NiTi surfaces. The elution test results could suggest a conformational change of fibronectin as a function of chemical composition of NiTi material and of surface treatment. Finally, a linear correlation between the amount of adsorbed albumin and the polar component of the surface energy of NiTi surfaces has been demonstrated. This work indicates that the OT treatment has an influence on the surface energy value of NiTi materials, which in turn influences the protein adsorption process.

Keywords: Nickel–titanium alloys; Protein adsorption; Surface energy; Fibronectin; Albumin

1. Introduction

NiTi shape memory alloys (SMA) are materials widely used in numerous biomedical applications (orthodontics, cardiovascular, orthopaedics, urology, etc.) [1,2] due to their unique shape memory properties and good corrosion resistance [3,4]. However, NiTi materials are still controversial biomaterials because of their high Ni content and the risks of allergy and adverse reactions that Ni release can trigger [5,6].

In order to reduce Ni surface concentration, a new oxidation treatment (OT) has been developed in a previous work [7]. It is a thermal oxidation, performed under low oxygen pressure to avoid Ni oxidation, that leads to the formation of a pure stochiometric dioxide titanium (TiO_2) on NiTi surface, as demonstrated by X-ray photoelectron spectroscopy (XPS) [7]. This TiO_2 oxide has been shown to efficiently protect NiTi surface from Ni ion release into the exterior medium and, therefore, may avoid toxic reactions associated with Ni.

However, the biocompatibility of this new surface treatment is a surface-related property associated with the biological interactions between the biomaterial surface and the biological medium. In particular, the interactions between the protein layer, adsorbed on the biomaterial surface immediately after its implantation, and the living cells in contact with it have been implicated in determining the biological response [8,9]. The cell response can drastically vary, depending on the amount, nature and conformation of the proteins adsorbed on the surface [10,11]. Presently, the mechanisms involved in the long-term biological response are not fully understood. However, it is known that some surface properties, particularly surface energy, have an influence on the protein adsorption process, and therefore on cell response. Various authors

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affirm that hydrophobicity enhances protein adsorption [12–16]. According to different works [17], this could be explained by the fact that the displacement of water molecules adsorbed on hydrophilic surfaces represents a substantial energetic barrier for protein adsorption. However, this is not a universal rule and it depends on the protein [18] and the specific surface. Generally, the protein adsorption on hydrophilic surfaces can be associated with water structure at the surface, electrostatic interactions and the conformational changes of the protein leading to more points of surface contact. Fibronectin, one of the key proteins involved in cell adhesion mechanisms [9,19–24], is an example of proteins that is able to adsorb on many hydrophilic surfaces [12,14,25], even if it prefers hydrophobic ones [12,26]. However, it has been shown that the adhesive functions of fibronectin decrease when it is adsorbed on hydrophobic surfaces [12,14,25].

There are very few studies available in the literature on protein adsorption on NiTi materials [27]. One of them, from Shabalovskaya [28], suggested that the amount of albumin adsorbed on NiTi should be proportional to the Ni surface content of the alloy. However, none of them has established a correlation between the protein adsorption and the surface energy of NiTi surfaces.

The aim of this work is, on one hand, to study the competitive protein adsorption process between albumin and fibronectin on untreated and oxidized (OT) NiTi surfaces. And, on the other hand, to analyze the influence of surface energy on the protein adsorption process on NiTi alloys. Indeed, we establish here a correlation between the surface energy values of NiTi surfaces and amount of protein adsorbed.

2. Materials and methods

2.1. Specimens

Cylinders of nearly equiatomic NiTi shape memory alloys (SMA) were obtained by threefold fusion in an argon atmosphere furnace. They were homogenized in a tubular furnace in an argon atmosphere at 1100 °C for 6 h, with a heating ramp of 6 °C/min. Disks of 8 mm in diameter and approximately 2 mm in thickness were cut from these cylinders. They were subjected to a thermal treatment to stabilize the β-phase and activate the shape memory properties. This treatment was previously optimized in our laboratory and consisted in heating at 800 °C for 10 min, quenching in cold water, and annealing at 500 °C for 1 h.

The samples were abraded with successive grades of SiC papers from 120 to 1200 grit and then polished with 1 μm alumina particles on Buehler® microcloth to obtain a mirror-like surface finish. A preliminary cleaning procedure was applied in all the samples, consisting of ultrasonical cleaning in a bath of acetone and a bath of distilled water, successively and for 10 min each. Prior to each experiment, additional cleaning and surface preparation was carried out.

Three different NiTi alloys obtained by the method previously described were studied. These alloys are denominated Ale1, Ale12 and Ale27 and have a corresponding Martensite start temperature (Ms) of 72, 56 and 11 °C, respectively. The Ale1 and Ale12 are of martensitic phase, whereas the Ale27 is of austenitic phase. The Ni content of these NiTi alloys varies as followed: Ale1 < Ale12 < Ale27, going from 49.5 to 50.5 at% approximately.

A grade II commercially pure Ti (c.p.Ti; Technalloy, S.L.) was also included in the study as a control material because of its well-known excellent biocompatibility.

Two different groups of materials were studied:

- Untreated materials: mirror-polished surfaces of NiTi (NT NiTi) and c.p. Ti;
- oxidized materials: NiTi alloys oxidized at 400 °C and 3×10^{-2} mbar, for 2 h 30 min (OT NiTi) (OT described in detail in [7]).

The apparent surface area of each sample was approximately 1.3 cm^2. The real surface area was calculated previously [7] by white light interferometer microscopy (NT 1100 model, Wyko), and was taken into account for the final protein adsorption results.

A minimum of three disks of each group were studied in all the different tests.

2.2. Contact angles and surface energy calculations

Two different liquids were used to carry out the contact angle measurements: ultrapure water (MilliQ®, Millipore™) and diiodomethane (Sigma).

The values of the polar and dispersive components of their surface tension are shown in Table 1.

Measurements of contact angles were carried out by the sessile-drop method with an OCA contact angle system (Dataphysics) at 25 °C.

The samples were ultrasonically cleaned in ethanol and in ultrapure water, successively, for 10 min each. The samples were dried with a pressurized flow of dried nitrogen, and used immediately. One sample was further placed into a hermetic chamber where the atmosphere was previously saturated with the liquid of measurement. A 3 μl droplet of the liquid was deposited on the surface of the sample, and immediately after stabilization, an image of the droplet was captured. The profile of the droplet was automatically fitted with the SCA20 software (Dataphysics) using a Young-Laplace approach. A minimum of ten values of contact angles were collected per each group of samples.

From the contact angle results obtained with the two liquids studied, the surface free energy (SFE) of each surface was calculated. The Owens and Wendt theory was applied (Eq. (1), [31]), and therefore, it was possible to obtain both polar, γ^p, and dispersive, γ^d, components of the SFE of each surface:

$$\gamma_L(1+\cos\theta) = 2\left((\gamma_L^d\gamma_S^d)^{1/2} + (\gamma_L^p\gamma_S^p)^{1/2}\right), \tag{1}$$

γ_L and γ_S are related to the liquid surface tension and solid surface energy, respectively.

Table 1
Values of the polar, γ^p, and dispersive, γ^d, components of the surface tension of the two liquids used in the study

Liquid	Dispersive component (γ^d, en mN/m)	Polar component (γ^p, en mN/m)
Ultrapure water [29]	21.8	51
Diiodomethane [30]	50.8	0