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Effect of nitinol wire surface properties on albumin adsorption

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Abstract

The superelastic, shape memory alloy nitinol (~50% nickel and ~50% titanium) is an important medical device material used for stent applications. However, the role specific surfaces properties have in protein adsorption remain controversial. In this study the effects of nitinol wire surface roughness, hydrophobicity and elemental composition upon albumin adsorption are investigated. In particular, we demonstrate that the technique of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the so-called surface mode can be used for the direct detection of albumin on the wire surfaces. In addition, albumin adsorbing to the wires was determined by using ^{125}I-labelled albumin. Albumin was detected on all wire samples. Surface roughness and hydrophobicity appeared to have no effect on albumin adsorption. There was however a clear correlation between the surface nickel and oxygen concentration and the amount of albumin adsorbed. Samples with higher levels of nickel and less oxygen in the surface oxide layer of the wires showed increased albumin adsorption, which could lead to improved biocompatibility. However, nickel is a toxic substance and can cause many adverse effects on humans, and thus nitinol with a slightly enriched surface nickel concentration that does not exhibit nickel release may have potential as a medical device material.

Keywords: Protein adsorption; Albumin; Nitinol; Surface-MALDI-TOF; Radiolabelling

1. Introduction

The adsorption of proteins onto biomaterial surfaces plays a key role in the way the surrounding body environment responds to the implanted material. Within seconds of implantation, protein adsorption onto the implant material will occur [1,2]. The proteins that spontaneously adsorb from the surrounding fluid medium onto the biomaterial surface after implantation are important in mediating the cellular response to the implant [3].

Albumin is important in biomedical applications as it has been identified on implants ex vivo [4]. The specific role that adsorbed albumin plays in responses to implants, however, remains controversial. While there is some evidence that shows that monocytes can and do adhere to albumin-coated surfaces in vitro, albumin is generally believed to "passivate" the surface and greatly reduce the acute inflammatory response [1,2]. A more recent study has shown that monocytes do not adhere to adsorbed albumin in vitro, but to macrophages via integrin receptors independent of the topography of the albumin. By topography we mean the two-dimensional structure of the adsorbed albumin layer, i.e. the formation of albumin clusters or a smooth monomolecular layer. The pro-inflammatory activity of macrophages was reduced on the albumin when compared to uncoated surfaces or surfaces coated with fibrinogen [5]. Albumin has been pre-adsorbed onto surfaces to suppress the non-specific adsorption of plasma proteins and concomitant cell–surface interactions [4]. A

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thin layer of albumin has been found to minimise adhesion and aggregation of platelets, thus reducing the possibility of subsequent thrombus formation [6]. Surfaces that have been coated with albumin have also been associated with lower bacterial adhesion [6]. Albumin also has a low conformational stability and because of this can adsorb onto a variety of hydrophobic or hydrophilic surfaces [7].

Albumin is the predominant plasma protein in blood and accounts for 60–70% of plasma. It has been reported that supramolecular organisation of the adsorbed protein layer is controlled by both surface chemistry and topography of the implant surface [8]. Despite this, it still remains unclear if surface chemistry and topography have an effect on protein adsorption, and if so what effect they have. At small micron level roughness it has been reported that albumin adsorbs preferentially to smoother surfaces than rougher surfaces [9]. Other studies reported that the adsorption of albumin is greatly increased on porous surfaces when compared to smooth surfaces [4]. Conflicting results have also been reported on the effect of surface hydrophobicity and hydrophilicity on albumin adsorption. One group has reported that the hydrophobicity of titanium has no effect on the adsorption of albumin [4], while other groups have found that if the hydrophilicity of titanium is increased, the extent of albumin adsorption decreased [9,10].

Alloys of nickel and titanium, and in particular nitinol (~50% Ni, 50% Ti), are of special interest in the medical device industry, due to their shape memory and superelastic properties. Superelasticity refers to the unusual ability of certain metals to undergo large elastic deformation [11]. The use of nitinol for medical purposes was first reported in the late 1960s [12], and today nitinol is commonly used for the manufacture of stents, which are primarily used in peripheral and coronary bypass graft interventions [13]. In the present study, the effect of nitinol wire surface roughness, hydrophobicity and elemental composition on the level of albumin adsorption is assessed. A variety of wires are used which contain varying amounts of oxygen and nickel in their oxide layers. It has been previously reported that albumin adsorption is higher on low oxygen containing surfaces [14]. A previous in vivo study has shown blood albumin levels to drop after 30 min at the site of nitinol implantation. This was believed to be due to the fact that albumin binds strongly to nickel [15]. However, since this was first proposed in 1987, no work has been carried out investigating the effect of nickel concentration on albumin adsorption.

In order to obtain reliable and usable data, it is important to use samples whose surface properties mirror those used in actual stent production. As with the majority of analysis carried out on biomaterials, flat coupons are the main sample choice when assessing protein adsorption on biomaterials [4,16,17]. The aim of the present study was to estimate the degree of albumin adsorption on nitinol wire samples. The initial detection of albumin on the wires was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the so-called surface mode, which is more commonly known as surface-MALDI-TOF. A detailed account of this method has previously been published [18]. To date, surface-MALDI-TOF has been used to analyse protein adsorption on polymeric materials, in particular contact lenses [18–21]. While this technique can be used to indicate whether proteins have adsorbed to surfaces or not, it provides little information on the amount of protein adsorbed. The most common method for quantitatively measuring protein adsorption onto surfaces is to use radioisotope labelled proteins [4,6,22]. This method is sensitive and reliable and it is the method that was used in this work to obtain quantitative data regarding albumin adsorption on nitinol wires.

2. Materials and methods

2.1. Materials

All nitinol samples used in this study were prepared by Fort Wayne Metals Research Products Corporation, IN, USA, specifically for this study. Nitinol wire samples with a diameter of 0.762 mm were fabricated from a binary nickel–titanium alloy with a nominal composition of 50.8 at.% nickel and an austenite start temperature in the fully annealed condition of −31 °C as measured by DSC per ASTM F 2004-00. Standard reduction and thermal processing was used to draw the wire to 1.02 mm. Additional processing to achieve 45% cold work at 0.762 mm followed by a heat straightening step to produce superelastic properties at room to body temperature were performed. The active austenite finish (A_f) temperature of the final wire was measured using the bend and free recovery method per ASTM-F 2082-01 to confirm that a superelastic condition had been achieved.

All specimens were cold drawn using either synthetic polycrystalline (Syn.Poly.) diamond dies or single crystal natural diamond (ND) dies. Heat straightening was performed at 500 °C under various levels of an argon/oxygen atmosphere. Various levels of the argon/oxygen atmosphere were used to produce samples with varying oxide thickness. After heat straightening, two of the wires were subjected to additional chemical and mechanical treatments in order to achieve the desired surface states. Removal of the oxide by etching (E) using a proprietary Fort Wayne Metals acid solution was performed on one specimen with the intent of attacking only the oxide itself. Another specimen was exposed to a pickling process (P) after the initial etching, again using an acid solution of a proprietary nature to Fort Wayne metals. This second chemical exposure allowed attack of the base material. A second sample that was pickled was then mechanically polished (M) using a mechanical wire polishing machine fixed with abrasive pads. After treatment, samples were kept in normal atmosphere conditions. Details of the wire surface preparation procedures are outlined in Table 1. All samples