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Fibre laser treatment of martensitic NiTi alloys for load-bearing implant applications: Effects of surface chemistry on inhibiting *Staphylococcus aureus* biofilm formation

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ABSTRACT

Biofilm infection is one of the main reasons for implant failure. It is extremely difficult to cure due to its high resistance to antibiotic treatments, and can result in substantial healthcare costs. In this study, the important shape memory NiTi alloy, in its martensitic state, was laser-treated using our newly-developed surface modification technique, aiming to tackle the biofilm infection problem. Martensitic NiTi was chosen for investigation because of its potential advantages in terms of (i) lower elastic modulus and (ii) higher damping capacity over its austenitic counterpart, giving rise to a lower risk of stress shielding and maximum stress between bones and load-bearing implants. The surfaces after laser treatment were systemically analysed using a series of surface measurement (i.e. surface roughness and water contact angle) and material characterisation (i.e. SEM-EDX, XRD and XPS) techniques. The antibacterial performance of the laser-treated surfaces was evaluated using the *Staphylococcus aureus* (or *S. aureus*) cells in-vitro cultured at 37 °C for 24 h. Fluorescence microscopy accompanied by Live/Dead staining was employed to analyse the cell culture results. The surfaces in their as-received states and after polishing were also tested and compared with the laser-treated surfaces in order to gain a deeper insight in how different surface conditions would influence biofilm formation. Our results indicate that the surfaces after laser treatment can mitigate bacterial attachment and biofilm formation effectively. The antibacterial performance was mainly attributable to the laser-formed oxides which brought desirable changes to the surface chemistry of NiTi. The laser-induced changes in surface roughness and topography, on a micrometre scale, only played a minor role in influencing bacterial attachment. The findings of this study demonstrated for the first time that martensitic NiTi with laser treatment could be a promising choice for the next-generation implants given its superior antimicrobial resistance and favourable mechanical properties for loading bearing applications.

1. Introduction

The unusual shape memory effect (SME) and super-elasticity (SE) coupled with good corrosion resistance, magnetic resonance compatibility and biocompatibility have made the near-equiatomic nickel titanium (NiTi) alloy a very popular choice in the medical device industry in the last decade. A medical devices market research report has shown that the global NiTi medical devices market for final medical components (namely stents, guidewires and others) was valued at US$ 8.2 billion in 2012 whilst the global market for the NiTi semi-finished goods (namely tubes, wiring, sheets and ribbons) was amounted to US$ 1.5 billion in 2012 [1]. The biomedical applications of NiTi span from the fields of orthodontic to orthopaedic, and vascular to neurosurgical applications [2, 3].

Both the SME and SE are direct consequences of the reversible martensitic transformation between Austenite (a high-temperature phase) and Martensite (a low-temperature phase) of NiTi [4]. There are four important temperatures associated with the martensitic transformation process, namely A_s (Austenite start), A_f (Austenite finish), M_s (Martensite start) and M_f (Martensite finish) temperatures. SME is a thermal-induced transformation process which refers to the ability of a deformed martensitic NiTi (i.e. undergoes deformation at $T < M_f$) to restore to its original shape in the austenitic state upon heating (at $T > A_f$), whilst SE is a stress-induced martensitic transformation

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process (i.e. keeps the material at T > A_f throughout the process), allowing the austenitic NiTi to sustain up to 8% strain without plastic deformation [4]. The majority of the NiTi biomedical applications stem from utilising the SME (i.e. used in the martensitic phase at room temperature but transforms to Austenite around the body temperature of 37 °C) or SE (i.e. used in the austenitic phase at 37 °C) [2, 3]. In other words, NiTi medical devices are used in the austenitic state at the human body temperature to actuate either the SME or SE.

Interestingly, NiTi in its martensitic state possess a desirable characteristic of very low elastic modulus (between 26 and 48 GPa) [5] which is much lower than that in its austenitic state (about 83 GPa) [5] and much closer to that of the cortical bone (between 19 and 21 GPa) [6]. The Martensite of NiTi can be thermally-induced or stress-induced. The martensitic NiTi mentioned herein refers to the thermally-induced Martensite. Martensitic and austenitic NiTi possess completely different mechanical transformation behaviours. Along with biocompatibility and osseointegration as well as wear and corrosion resistance, elastic modulus is one of the most important selection criteria of load-bearing implants in bone plates for internal fixation. Large mismatching of elastic modulus between the implant material and the adjacent cortical bone (i.e. particularly if the material is stiffer than the bone, then the implant will bear more of the load) can contribute to "stress shielding effect", which causes the subsequent bone resorption and aseptic loosening (which accounts for nearly 30% of implant failure rate for revision) [7]. In addition, martensitic NiTi is well known for its high damping capacity [8]. The lower elastic modulus and higher damping capacity would result in (i) lower risk of stress shielding, (ii) more absorption of impact energy and (iii) lower maximum stress between the bones and implant [9]. Higher resistance to low-cycle fatigue [10] and comparable corrosion resistance to austenitic NiTi [11] are the additional benefits of martensitic NiTi. From the manufacturing point of view, martensitic NiTi is advantageous over austenitic NiTi because of ease in shape forming and machining. However, existing research in martensitic NiTi is significantly less than its austenitic counterpart. This paper serves as one of the first initiatives to explore and report the possibility of using martensitic NiTi in biomedical applications.

The high nickel (Ni) content in NiTi poses a risk of the release of harmful Ni ions into body fluids and tissues in contact. It is known that the outermost Ti oxide layer on the NiTi surface acts as a barrier to confine the diffusion/release of Ni ions [12, 13]. The quality and protectiveness of the oxide layer are controlled by several attributes: composition, oxide species, oxide uniformity and homogeneity [14]. These attributes of the oxide layer depend on the previous manufacturing history (e.g. mechanical polishing, chemical passivation or etching, and heat-treatment) [13, 15] and can be varied by post-process surface treatments (e.g. ion implantation [16, 17], thermal oxidation [18, 19] and laser treatment (LT) [15, 20–25]).

Among the aforementioned post-process techniques, LT including laser surface melting, nitriding and oxidation techniques offer the promising capabilities of precisely tailoring the surface characteristics (namely microstructure, chemistry, roughness and topography) in local areas as well as being accurate, repeatable and clean (a non-contact process). Positive effects of LT to reduce Ni release have been documented in literature. The improvements mainly stem from the following two characteristics related to the laser-formed oxide layer at the outermost surface: (i) increased percentage (at.%) of TiO_2 in the oxide layer [23] and (ii) enhanced oxide homogeneity/uniformity as a result of surface homogenisation after LT (i.e. removal of the pre-existing surface inclusions and/or Ni-rich regions in the base material [15]). Moreover, the other beneficial characteristics of the laser-formed oxide layer such as reduction of surface metallic state [22–25] and oxide thickening [20] contribute to the improvement in corrosion resistance. In addition, the present author found that low Ni metal/TiO_2 ratio in the oxide layer together with the laser-induced physical changes in roughness and topography can promote better cell-surface interactions (i.e. higher attachment and viability of mesenchymal stem cells (MSCs) compared to the untreated NiTi [26]).

Taken together, the focus of existing literature on the laser-formed oxide layer on NiTi after LT has been on the evaluation of corrosion, Ni ion release and biocompatibility (i.e. formation of bone-like apatite *or* in-vitro culture of bone cells: osteoblasts or MSCs) performances. It is important to point out that biofilm infection is one of the main reasons for implant failure accounting for about 15% of failure (i.e. ranked second to the failure caused by aseptic loosening) [7]. Biofilm infection is extremely difficult to cure due to its high resistance to antibiotic treatments and can result in substantial healthcare costs due to the need to remove the infected foreign body to cure infection [27, 28]. A biofilm-mediated infection starts with initial bacterial adherence to a surface, followed by growth, replication, and production of protective extracellular substances which shield the resulting sessile bacterial community. Initial reversible bacterial adherence starts within the first few seconds and extends to approximately 2 h, after which adherence becomes irreversible [29]. Accordingly, bio-fouling by bacteria is much faster than the corrosion and Ni ion release processes (usually take days to months to occur). Bacterial adherence can interfere with the tissue regeneration processes on the implant surfaces [30], and also exacerbate the corrosion behaviours of NiTi [31] thus worsening the Ni ion release problem. Surface chemistry is known to be a key factor, along with hydrophobicity, surface roughness and topography, to impact the initial adhesion and aggregation of bacteria (e.g. *Staphylococcus epidermidis* [32], *Staphylococcus aureus* [33], and *Escherichia coli* [34]). Up to the present, no effort has been devoted to investigating the effectiveness of the laser-formed oxide layer on inhibiting the bacterial adherence and biofilm formation on NiTi surface after LT.

In this study, martensitic NiTi alloy were laser-treated using the newly-developed LT technique [33, 35, 36]. This LT technique is designed to operate in an open air environment with the treatment area protected by coaxially delivered Ar, and hence no additional gas chamber and tubes are required. It can be applied to treat the free-form, complexly-shaped surfaces that are commonly seen in biomedical implants. The major objective of this study was to investigate the surface chemistry effects of NiTi after LT on inhibiting the in-vitro bacterial adherence and biofilm formation of *S. aureus*. The effects of other laser-induced surface characteristics: surface roughness, topography and wettability were also analysed and systemically compared with the two untreated NiTi samples, namely as-received and mechanically-polished surfaces.

2. Methodology

2.1. Material preparation

The near-equiatomic NiTi (55% Ni, balance Ti by weight) alloy used in this study was sourced from American Elements (Los Angeles, California, USA). The NiTi material was purchased in form of a plate with the size of 250 mm × 250 mm and thickness of 2 mm. The samples, in form of circular discs of 15 mm in diameter, were cut from the NiTi plate using the electrical discharge machining (EDM) wire-cut method. Before laser treatment, the sample surfaces were sequentially ground with a range of sandpapers from 120 to 400 grit to ensure the consistency in surface roughness and to remove the pre-existing surface oxide layer. The disc samples after grinding were cleaned in an ultrasonic bath filled with ethanol for 10 mins, then rinsed in distilled water for 10 mins and eventually dried completely in air.

2.2. Thermal transformation analysis

The transformation temperatures of the NiTi sample, namely Martensite start (M_s), Martensite finish (M_f), Austenite start (A_s) and Austenite finish (A_f) were measured using a differential scanning calorimeter (DSC – Pyris™ Diamond, PerkinElmer Instruments, Waltham, MA, USA). The temperature range scanned in the DSC analysis was