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The biocompatibility and mechanical properties of cylindrical NiTi thin films produced by magnetron sputtering

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ABSTRACT

Superelastic nickel titanium shape memory alloys (NiTi–SMA) are of biomedical interest due to the large obtainable strains and the constant stress level. Production of NiTi–SMA thin films by magnetron sputtering was developed recently. NiTi sputtered tubes have a high potential for application as vascular implants, e.g. stents. Magnetron sputtering, three dimensional lithography and wet etching were used in order to produce Ti and NiTi stent devices (thickness: 5–15 μm; diameter: 1–5 mm). For tensile tests, specimens were prepared in radial and axial directions in order to compare the mechanical properties of the film in both directions. The specimens – produced for cell culture experiments – were incubated with human mesenchymal stem cells (hMSC) for 7 days. Cell viability was analyzed via fluorescence microscopy after live/dead staining of the cells. Cytokine release from cells was quantified via ELISA. Cylindrical NiTi films showed a strain up to 6%. Tensile parameters were identical for both directions. Best material properties were obtained for deposition and patterning in the amorphous state followed by an ex-situ crystallization using rapid thermal annealing in a high vacuum chamber. First biological tests of the Ti and NiTi–SMA samples showed promising results regarding viability and cytokine release of hMSC.

1. Introduction

Nickel–titanium shape memory alloys (NiTi–SMA) exhibit mechanical and chemical properties which make them attractive candidate materials for various types of biomedical applications (e.g., shape memory staples for compression osteosynthesis, hip endoprosthesis and acetabular cups with integrated self-expanding NiTi elements) [1]. These alloys demonstrate good deformability that is associated with their supposed "pseudoelastic" behavior. A mechanically imposed strain as high as 8% can be reversibly recovered after unloading. NiTi is well suited for applications in the medical field since the material is known for its excellent biocompatibility [1]. The pseudoelastic properties are often needed at 37 °C which can be achieved by choosing an appropriate slightly Ni-rich composition $Ti_{50-\varepsilon}Ni_{50+\varepsilon}$ ($\varepsilon \approx 0.7$) and by thermal treatment or a certain amount of cold work. Another typical application for NiTi–SMA is endovascular implants [2]. The fabrication of small but flexible NiTi tubes is a particular difficult task and only a relatively large homogeneous wall thickness exceeding 50 μm can be reached when using bulk materials. Another problem also gains importance when producing NiTi stents with relatively thin walls. Standard Nitinol material contains high numbers of inclusions (TiC) within the microstructure and it is frequently observed that fatigue cracks tend to initiate at inclusions [3]. FEM simulations for stents revealed that the TiC inclusion itself greatly changes the stress distribution. The maximum stress level of a stent model with TiC defect is about ten times as large as the structure without defect. It can be concluded that cracks/fractures are likely to occur near the defect [4]. In smaller stents, the relative size of inclusions increases with decreasing strut diameter. To fabricate endovascular devices for smaller vessels the application of thin film techniques is considered to be an attractive alternative approach [5]. The success of stents and other medical implants based on superelastic NiTi, which are produced by conventional thin-walled tubes and then structured with the laser [6] and its limitations regarding, miniaturization, have motivated the fabrication and structuring of stents based on thin film technology. In recent years, NiTi material miniaturization is in trend and the need for semi-finished products and tubes on the basis of thin film techniques is clear to observe. This work covers the fabrication of such NiTi thin film structured tubes by using three techniques: 1) magnetron sputtering of superelastic NiTi [7,8], 2) three dimensional UV photolithography [9] and 3) wet etching. The main objective of the experiments described here was to assess whether magnetron sputtering is a suitable method to produce NiTi thin film stents. Therefore biocompatibility, including cell viability and cytokine release, was analyzed. We used human mesenchymal stem cells (hMSC) as a cellular model. HMSC can be differentiated in vitro into osteoblasts [10], chondrocytes [11,12], tenocytes [13], myocytes [14] and adipocytes [11]. It was also shown that MSC transdifferentiate toward an endothelial cell phenotype [15].

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HMSC can be easily expanded in vitro and retain their developmental potential during extensive cultivation steps or cryopreservation [16]. Therefore, hMSC are ideal cells for the application as a cellular model.

2. Materials and methods

2.1. Magnetron sputtering

All fabrication steps were performed in the clean room facility "Kieler Nanolabor". An Alcatel 450 (DC) magnetron sputtering device was used to produce NiTi thin films. NiTi 53.2 at.% targets were manufactured by cast melting at the Mechanical Engineering Department at the University Bochum [17]. The base pressure of the vacuum chamber was approximately 1×10^{-7} mbar. Thin films were sputtered on glass substrates by employing a specific device which allows an in-situ rotation of the substrate during the sputtering process. Freestanding thin films were obtained by pre-sputtering thin sacrificial layers in the following sequence: glass (substrate) + multilayer Cr/Cu. Thin films of NiTi were deposited on unheated substrates that result in an amorphous film. Subsequently, the samples were crystallized in a high vacuum chamber in order to avoid oxidation during the annealing process. The heat treatment was carried out ex-situ by means of a rapid thermal annealing (RTA) system. The halogen lamp driven heating chamber enables typical heating ramps of 50 K/s in a vacuum environment of about 10^{-6} to 10^{-7} mbar. The annealing temperature was held constant for 10 min at the maximum temperature of 650 °C, which turned out to be sufficient for crystallization of amorphous NiTi. In a second step, films were annealed for further 10 min at 450 °C, which is a common procedure for Ni-rich samples to induce the formation of Ti_3Ni_4 precipitates in order to adjust the conversion temperatures of the martensite to austenite phase transformation.

2.2. Three dimensional photo lithography

After the sputtering process, the film was dip coated with photo resist AZ1518 of 2 μm thickness. The soft bake was performed on a hotplate at 105 °C for 2 min followed by rehydration in a controlled atmosphere for another 2 min. A chromium covered quartz mask with a feature resolution of 500 nm was used for the photolithography. Fig. 1A shows a design used for biocompatibility tests while Fig. 1B shows a design used for tensile tests. The exposure time was 2 s and the resist was developed using an AZ726 MIF solution for 30 s. The exposure was carried out on a standard Suess (MJB4) Microtec lithography device which was adapted to operate in combination with a self-developed rotational device (Fig. 2). The rotational device was constructed in order to expose substrates from 0.5 mm to 5 mm diameter using two linear motors, a rotational one with 0.01° resolution and a linear with 6.3 μm resolution mounted on an x–y linear table. A substrate holder allows a vertical adjustment of the cylindrical substrate in relation to the Cr mask with a precision of 1 μm. This vertical adjustment allows the exposure of substrates with different diameters and is an important parameter to define the feature size resolution as shown in the results published in our previous work [9]. Three dimensional photo lithography is based on the synchronized movement between the planar Cr mask and the substrate as shown in Fig. 2, where V_m is the Cr mask speed, S_w shutter width, f_s rotational frequency of cylindrical substrate and d_{out} outer diameter of cylindrical substrate.

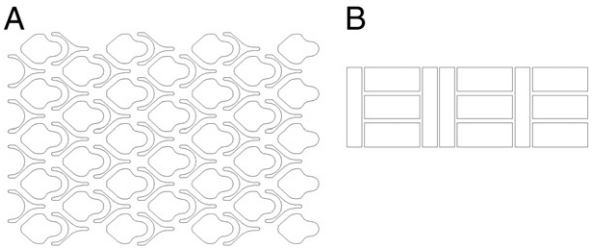

Fig. 1. Design of the chromium covered quartz masks which were used for the photolithography. (A) Design used for biocompatibility tests, (B) design used for tensile tests (dimensions 11×3 mm).

2.3. Wet etching

After the exposure of the photo resist the film is wet etched. Fig. 3 shows the entire processes flow, from sputtering to wet etching. The sample is placed on a HF solution (HF, HNO_3, $NiSO_4$ and H_2O—6%/25%/6%/63%) for 12 min. After this step the sacrificial layers are wet etched using a standard BASF Selectipur Chromium Etch, an ammonium cerium (IV) nitrate based etchant, which does not etch TiNi thin films.

2.4. Tensile test

Tensile testing of freestanding films was performed at 37 °C in a universal tensile test device (Messphysik UTM Beta EDC 100N) with the sample being located within a temperature chamber (Shimadzu TCL-N382). A pre-load of 0.2 N is applied in order to assure that the film is pre-stretched and well fixed. A constant strain speed of 0.4%/min is applied.

2.5. Cultivation of human mesenchymal stem cells

In order to analyze the biocompatibility of NiTi specimens, hMSC were cultured on NiTi specimens. HMSC (3rd–6th passage) obtained from LONZA (Walkersville Inc., MD, USA) were grown in cell culture flasks (Falcon, Becton Dickinson GmbH, Heidelberg, Germany) using RPMI1640 medium (GIBCO, Invitrogen GmbH, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS, GIBCO, Invitrogen GmbH) and L-glutamine (0.3 g/l, GIBCO, Invitrogen GmbH) at 37 °C in a humidified atmosphere containing 5% CO_2. Cell culture conditions were maintained throughout the experiment. For cell passage or harvest, adherent cells were detached from the culture flasks by the addition of 0.2 ml/cm^2 0.25% trypsin/0.05% ethylendiamine tetraacetic acid (EDTA, Sigma-Aldrich, Taufkirchen, Germany) for 5 min at 37 °C. Cells were collected, washed twice with RPMI/FCS, resuspended in RPMI/FCS, and seeded on the specimens.

2.6. Reaction of cells

2.6.1. Viability

To assess whether magnetron sputtering is a suitable method to produce NiTi thin film stents, live/dead staining in combination with fluorescence microscopy was used [18] to document cell viability. In live/dead staining, living cells give rise to a green fluorescence while dead cells appear red. After 7 days of cell culture, the cells were incubated with calceinAM (50 ng/ml, Calbiochem, Schwalbach, Germany) for 30 min at 37 °C using cell culture conditions. Subsequently, samples were washed with cell culture medium RPMI1640 followed by staining with propidium iodine (50 μg/ml, Sigma-Aldrich, Taufkirchen, Germany) for 15 min at room temperature while protected from light. After twofold washing with RPMI1640 the stained hMSC were analyzed using light and fluorescence microscopy (Olympus MVX10, Olympus; Hamburg, Germany).

2.6.2. Cytokine release

Interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) released from hMSC were quantified in the cell culture supernatants using enzyme linked immunosorbant assays (ELISA). Antibodies, as