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Cell adhesion on NiTi thin film sputter-deposited meshes

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ABSTRACT

Scaffolds for tissue engineering enable the possibility to fabricate and form biomedical implants in vitro, which fulfill special functionality in vivo. In this study, free-standing Nickel–Titanium (NiTi) thin film meshes were produced by means of magnetron sputter deposition. Meshes contained precisely defined rhombic holes in the size of 440 to 1309 μm^2 and a strut width ranging from 5.3 to 9.2 μm. The effective mechanical properties of the microstructured superelastic NiTi thin film were examined by tensile testing. These results will be adapted for the design of the holes in the film. The influence of hole and strut dimensions on the adhesion of sheep autologous cells (CD133+) was studied after 24 h and after seven days of incubation. Optical analysis using fluorescence microscopy and scanning electron microscopy showed that cell adhesion depends on the structural parameters of the mesh. After 7 days in cell culture a large part of the mesh was covered with aligned fibrous material. Cell adhesion is particularly facilitated on meshes with small rhombic holes of 440 μm^2 and a strut width of 5.3 μm. Our results demonstrate that free-standing NiTi thin film meshes have a promising potential for applications in cardiovascular tissue engineering, particularly for the fabrication of heart valves.

1. Introduction

The development of new scaffolds for tissue engineering is a challenging task that has become of increasing interest in the last decade. The mechanical properties of such scaffolds and their design are known to strongly affect cell growth, the formation and integration of extracellular matrix proteins and the functionality of the device as a whole [1]. A tissue engineering approach in general for generating biomedical implants has various promising applications. This is particularly the case for bioprosthetic cardiovascular implants. The major issues of conventional bioprosthetic heart valves, such as xenografts, are the limited durability due to re-calcification.

NiTi is a widely used material for many implant types such as stents, septal occluders or vena cava filters [2]. Due to its superelastic properties it is well suited for transcatheter-based implants. The implant regains its initial shape when it is deployed from the catheter so that no balloon expansion system is needed. Further studies proved the excellent biocompatibility of NiTi material [3–5]. NiTi thin film technology allows the fabrication of complex geometrical structures with micrometer precision from materials with high cyclic mechanical stability [6]. In this regard, NiTi has already demonstrated great potential as a biomaterial for heart valve leaflets [7,8].

Before applying such materials for in vivo applications, biocompatibility and cell growth must be guaranteed. It is a well-studied phenomenon that cell adhesion can be controlled geometrically by 2D micropatterns of extracellular matrix proteins. Such patterns are often generated either with microcontact printing [9] or by using bottom-up approaches [10,11]. On such protein micropatterns it is possible to control cell life and death by pattern geometry [12] and to influence cell shape, focal adhesion and actin stress fiber formation [13]. In addition, not only the size and micropattern of a cell-adhesive area are responsible for the ability of a cell to spread on a substrate, but also the nanoscale spacing of single cell adhesion ligands [14,15]. A further important role has been attributed to surface microtopography. Several studies have shown that cell adhesion, spreading and even tissue morphogenesis can be influenced by 2D micropillar structures [16]. Such micropillars can be fabricated from silicon elastomers and cell behavior is co-regulated by substrate topography and pillar bending stiffness [17,18].

Common approaches to generate materials for cardiovascular tissue engineering include cell seeded non-biological scaffolds as well as decellularized donor tissue. Decellularized native porcine heart valves seeded with CD133+ cells have shown good functionality in pulmonary valved stent implantation [19]. A further strategy to generate cardiovascular scaffolds by tissue engineering is the use of electrospun materials [20,21]. For example, Hinderer et al. studied the influence of electrospun poly-(L-lactide) scaffolds on valvular endothelial cells (VECs) and valvular interstitial cells (VICs) for bio-functionalized hybrid

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heart valves [22]. Electrospun scaffolds made from poly(glycerol sebacate) and poly(ε-caprolactone) are also materials suitable for generating tissue engineered heart valves [23].

Compared to such polymer based tissue engineering approaches, a sputtered thin film NiTi based scaffold has the advantage of well-defined geometrical structures, uniform hole sizes and controllable effective mechanical properties as well as thinner structures for the same mechanical integrity.

In a recent study, Alavi et al. have reported on a hybrid heart valve leaflet based on a superelastic NiTi mesh core [24]. The NiTi scaffold was 76 μm thick and the mesh structure was acid etched, leading to a network of circular holes with a diameter of 240 μm and a central distance of 320 μm. This scaffold could be completely enclosed with a multi-layer of biological tissue. This work proved the suitability of NiTi scaffolds for biological applications in general. An examination of the inflammatory response found a lower TNF-α level for tissue enclosed meshes than for bare NiTi meshes, and furthermore, the superior biocompatibility of NiTi compared to stainless steel was confirmed.

In this study we characterized NiTi thin film meshes that contain rhombic holes. Mechanical properties were strongly related to the size of the holes and struts in the mesh, as well as on their orientation. In order to investigate the biological impact of these NiTi meshes, the adhesion of autologous progenitor cells (CD133+) on different NiTi meshes was studied. Cells adhering to the meshes were investigated at two different time points after 24 h and after seven days of incubation, using fluorescence microscopy and scanning electron microscopy (SEM), demonstrating the biocompatibility of the samples and the dependence of cell growth on the hole and strut dimensions.

2. Materials and methods

2.1. Fabrication of structured nickel–titanium thin films

NiTi thin film samples were fabricated by means of magnetron sputtering with an Alcatel 450 sputtering device on a silicon substrate. In order to attain freestanding films, NiTi films were deposited on a pre-sputtered Copper (Cu) sacrificial layer. Sputtering was carried out at a base pressure below 1×10^{-7} mbar, an Argon flow of 20 sccm and a sputtering pressure of 2×10^{-3} mbar. The magnetron sputtered films have a composition of $Ni_{50.5}Ti_{49.5}$ atom %. UV lithography and wet etching were performed to pattern NiTi thin film meshes and to attain freestanding films. The detailed process flow chart of the process and the wet etching procedure were described by Lima de Miranda et al. [25].

Rapid thermal annealing (RTA) with a heating rate of 50 K/s was used at 650 °C for 10 min to crystallize the amorphous NiTi. In detail, RTA was carried out in a vacuum environment at a pressure of about 10^{-7} to 10^{-6} mbar to avoid oxidation of the samples during heating. An additional aging step at 450 °C for 10 min, which is a common procedure to induce Ti_3Ni_4 precipitates in order to adjust the austenite to martensite phase transition temperature was also applied. The resulting austenite finish temperatures of 26.4 ± 0.9 °C were determined by differential scanning calorimetry. Thin film samples were fabricated as so-called dog bone structures with a thickness of 10 μm, having a rhombic hole mesh structure in their central rectangle, which had a length of 4.5 mm and a width of 2 mm (Fig. 1). Three different mesh sizes were fabricated, which are denoted as small (S), medium (M) and large (L). Characteristic sizes of the meshes are presented in Table 1.

2.2. Tensile test

Mechanical tests were performed in a Zwick/Roell Z0.5 tensile test device at a temperature of 37 °C. In order to gain information on the tensile properties of the NiTi thin film scaffolds, samples were characterized with the long axis of the rhombic mesh structure oriented parallel and perpendicular to the pulling direction, respectively. Tensile tests were carried out at a constant strain rate of 0.8%/min.

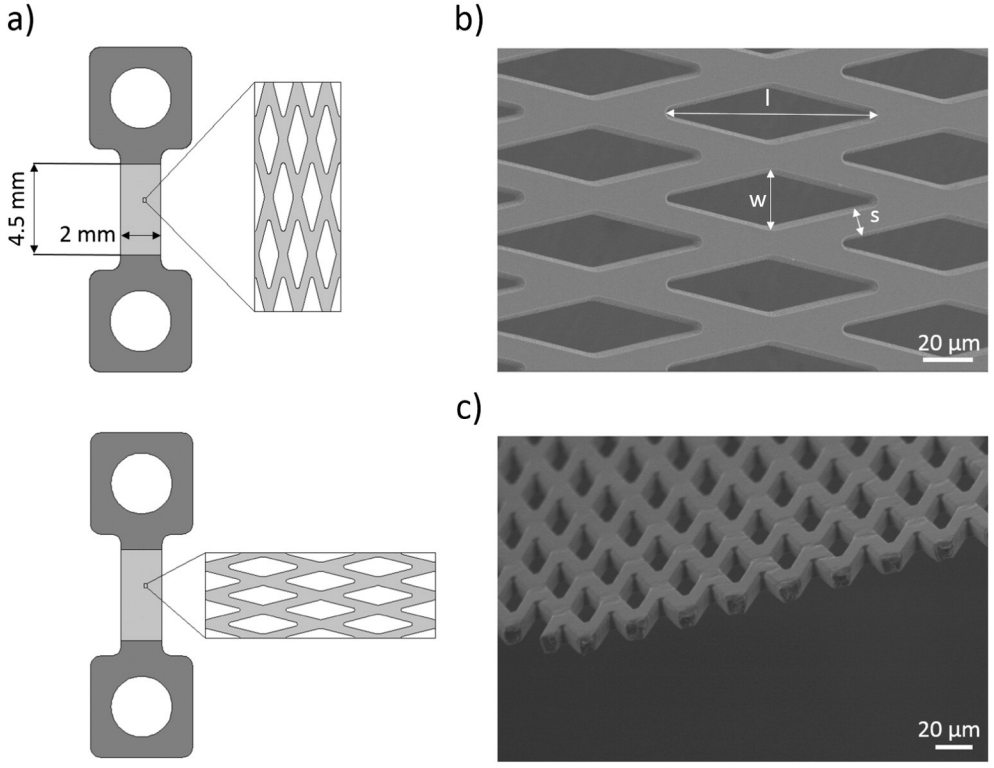

Fig. 1. Sample design: a) Transversally and longitudinally oriented rhombic dogbone structure with a parallel length of 4.5 mm and a width of 2 mm. b) Micrograph of the rhombic holes in the mesh with the rhombic length l, rhombic width w and the strut size s. Characteristic scaffold strut and rhombic hole sizes of freestanding NiTi meshes are given in Table 1. Thin film technology enables a mesh fabrication with precise rhombic hole and strut sizes throughout the whole scaffold. c) Fractured longitudinal NiTi thin film mesh.