Materials Science & Engineering C 93 (2018) 12–20

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Hierarchical structures on nickel-titanium fabricated by ultrasonic nanocrystal surface modification

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ARTICLE INFO

Keywords:
Hierarchical structures
Ultrasonic nanocrystal surface modification
Biomedical implant
Severe plastic deformation
Biocompatibility
Corrosion

ABSTRACT

Hierarchical structures on metallic implants can enhance the interaction between cells and implants and thus increase their biocompatibility. However, it is difficult to directly fabricate hierarchical structures on metallic implants. In this study, we used a simple one-step method, ultrasonic nanocrystal surface modification (UNSM), to fabricate hierarchical surface structures on a nickel-titanium (NiTi) alloy. During UNSM, a tungsten carbide ball hits metal surfaces at ultrasonic frequency. The overlapping of the ultrasonic strikes generates hierarchical structures with microscale grooves and embedded nanoscale wrinkles. Cell culture experiments showed that cells adhere better and grow more prolifically on the UNSM-treated samples. Compared with the untreated samples, the UNSM-treated samples have higher corrosion resistance. In addition, the surface hardness increased from 243 Hv to 296 Hv and the scratch hardness increased by 22%. Overall, the improved biocompatibility, higher corrosion resistance, and enhanced mechanical properties demonstrate that UNSM is a simple and effective method to process metallic implant materials.

1. Introduction

A good implant material needs hierarchical surface structures that can act as scaffold with multiscale dimensions for cell adhesion and growth [1,2]. Mammal bones, for example, consist of different dimensions of collagen fibrils and mineral apatite particles [3]. The collagen fibrils, assembled collagen molecules in the form of higher-order polymers, normally have micro- or submicroscale lamellar structures that function as scaffold for cell growth [4,5]. The nanoscale apatite particles nucleate and grow in the gap zone of collagen fibrils [6]. These hierarchical structures provide a matrix for cell attachment, promote cell proliferation and differentiation, as well as meet the mechanical requirements for bone tissue.

Inspired by natural hierarchical structures [7], many researchers used artificial hierarchical structures to enhance cell-matrix interactions in tissue engineering. It has been reported that materials with hierarchical structures can improve biocompatibility of biomaterials [8,9]. However, fabricating hierarchical structures on biomaterials, especially metallic implants, is challenging. Most methods used for the fabrication of hierarchical structures involve building a macroscale template followed by the creation of nanoscale structures [8,10]. During these procedures, more than one fabrication techniques such as photolithography [11–13], sol–gel method [14,15], acid treatment [13,16], anodization [17,18], *etc.*, are usually needed. For example, hierarchically ordered structures with multiple length scales were fabricated by combining micro-molding, polystyrene sphere templating, and cooperative assembly of inorganic sol-gel species with amphiphilic triblock copolymers [19]. These multi-step processes make fabrication

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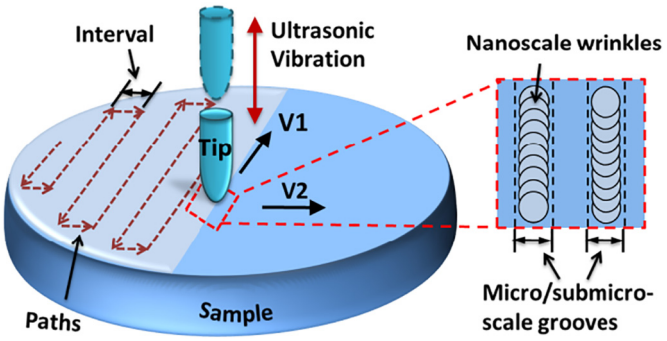
https://doi.org/10.1016/j.msec.2018.07.032
Received 11 October 2017; Received in revised form 7 June 2018; Accepted 12 July 2018



Scheme 1. Schematic of the UNSM process.

of hierarchical structures complex, costly, and time consuming. Additionally, for biomaterials, both biomedical and mechanical requirements should be considered. Therefore, it is desirable to fabricate hierarchical structures with a simple method, which at the same time can improve the mechanical properties of metallic implants.

In this study, we report a simple one-step method, ultrasonic nanocrystal surface modification (UNSM), to fabricate hierarchical surface structures on Nickle-Titanium (NiTi). During the UNSM process (Scheme 1), a tungsten carbide ball attached to an ultrasonic device scans over the sample surface while striking it at high frequency (20 kHz). UNSM can be used to fabricate hierarchical surface pattern by manipulating the process parameters including tip diameter, strike intensity, and strike density, all of which can be precisely controlled. In addition to hierarchical surface structures, the grain refinement induced by UNSM is believed to promote cellular activity [20–23] and to improve mechanical properties [24–30]. A previous study showed that three-pass UNSM can generate an amorphous surface layer on NiTi [31]. In this study, by fabricating hierarchical surface structure on NiTi using UNSM with carefully selected process parameters, not only was the biocompatibility of the NiTi alloy enhanced, but also the corrosion performance and the mechanical properties were significantly improved.

2. Experimental section

2.1. Material preparation

NiTi (50.4 at.% Ni, thickness 1.5 mm, active A_f 54.6 °C) plates from Special Metals (WV, USA) were cut into disks with diameters of 15 mm. Disk samples ground up to 1200 grit with silicon carbide grinding paper (Buehler, Microcut®, 8 in.) were used as control. For the UNSM group, the disk samples were ground up to 1200 grit followed by UNSM treatment. For the cell culture study, the control samples were ground up to 800 grit to reduce the difference in surface roughness between the control and the UNSM groups.

In this study, the UNSM experiment was carried out with the following conditions: a static load of 20 N, a vibration amplitude of 12 μm, a scanning speed (Scheme 1, V_1) of 1000 mm/min and an interval (distance between neighboring scans) of 10 μm. The details of the process paths are shown in Scheme 1. In this study, the diameter of the UNSM-treated area was set as 15 mm, leaving an annulus with ~0.2 mm width on the edge not treated.

2.2. Surface morphology

The surface morphology of samples before and after UNSM was characterized using a Zygo NewView 7300 surface profiler. The scanning area was 0.18 mm × 0.13 mm for 400× magnification. Each scanning area generated a roughness average, R_a, of the surface, and five measurements were carried out to obtain the mean R_a. A scanning electron microscope (SEM) (LYRA3, TESCAN) operating at 20 kV was also used to characterize surface morphology.

2.3. Microstructure

Cross-sections of the UNSM samples were ground up to 1200 grit with silicon carbide grinding paper before etching. After etching (with nitric acid and hydrofluoric acid at volume ratio of 3:5) for 1 to 2 s at room temperature, samples were immediately rinsed with flowing water. The microstructures were characterized with a digital optical microscope (MU130, AmScope).

2.4. In vitro cell viability

To evaluate the effect of UNSM on cell survivability, cytotoxicity study was carried out using a LIVE/DEAD cell viability assay. At first, three samples from the control group and the UNSM group were ultrasonically cleaned in dichloromethane, acetone, ethanol, deionized water, and ethanol again for 5 min each, using a 3510 Branson ultrasonic cleaner with an ice-water mixture bath, followed by sterilization by ultraviolet radiation in a biosafety cabinet for 12 h. Adipose-derived stem cells (ADSCs, from Lonza®, Switzerland) at passage 5 were cultured in a regular growth medium (α-MEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin/streptomycin) at 37 °C in a 5% CO_2 atmosphere for 72 h. After that, cells were harvested, seeded, and incubated on the cleaned samples that were already transferred into 24-well cell culture plates. Cells within the regular growth medium were seeded at 5000 cells/cm^2. After incubation for 24 h, 1 ml combined LIVE/DEAD cell staining solution (2 μM calcein AM and 4 μM EthD-1 in phosphate-buffered saline (PBS)) was added to each well and incubated for 5–10 min at room temperature. Images were recorded using an IX51 Epifluorescence microscope (Olympus Co., Japan) equipped with a fluorescent light source and filters. The viability was calculated by manually counting the number of live cells and dead cells. Five randomly selected images from each group were used for this calculation. This part of cell culture experiment was repeated twice.

To examine cell adhesion and spread, cell patterns were examined using Millipore's Actin Cytoskeleton and Focal Adhesion Staining Kit (FAK100). At first, after the aforementioned cleaning and sterilizing steps, disk samples were transferred into 24-well plates, followed by seeding ADSCs on the 24-well plates at 5000 cells/cm^2 and culturing for 48 h. Then, the cells were washed with PBS and fixed in 5% paraformaldehyde in PBS (pH 7.4) for 15 min at room temperature. After the blocking solution was aspirated, the cells were washed with PBS twice, followed by treatment with anti-vinculin monoclonal antibody (diluted 1:500) for 1 h at room temperature for vinculin staining. Next, TRITC-conjugated phalloidin (diluted 1:1000), in combination with a FITC-tagged secondary antibody (diluted 1:1000), were used for 30 min at room temperature for F-actin cytoskeleton staining. Finally, nuclei were stained by DAPI (diluted 1:1000) for 5 min at room temperature. Cells were washed three times with PBS after each staining. Finally, the stained cells were imaged with the same IX51 Epifluorescence microscope. The area per cell and the ratio of cell length to width from the two groups were obtained after randomly counting ~30 cells from three images of each group with the ImageJ software. This part of cell culture experiment was repeated three times.

2.5. Corrosion test

The corrosion behavior of NiTi samples was investigated using electrochemical testing, including open circuit potential (OCP), potentiodynamic polarization (PDP), and electrochemical impedance spectroscopy (EIS). All electrochemical measurements were carried out using a VersaSTAT4 electrochemical workstation (Princeton Applied Research) at room temperature. A standard three-electrode flat cell was used. The reference, counter, and working electrodes were a saturated calomel electrode (SCE, $Hg/Hg_2Cl_2/KCl$), a platinum mesh, and a test