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Understanding the impact of grain structure in austenitic stainless steel from a nanograined regime to a coarse-grained regime on osteoblast functions using a novel metal deformation–annealing sequence

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ABSTRACT

Metallic biomedical devices with nanometer-sized grains (NGs) provide surfaces that are different from their coarse-grained (CG) (tens of micrometer) counterparts in terms of increased fraction of grain boundaries (NG > 50%; CG < 2–3%). The novel concept of 'phase-reversion' involving a controlled deformation–annealing sequence is used to obtain a wide range of grain structures, starting from the NG regime to the CG regime, to demonstrate that the grain structure significantly impacts cellular interactions and osteoblast functions. The uniqueness of this concept is the ability to address the critical aspect of cellular activity in nanostructured materials, because a range of grain sizes from NG to CG are obtained in a single material using an identical set of parameters. This is in addition to a high strength/weight ratio and superior wear and corrosion resistance. These multiple attributes are important for the long-term stability of biomedical devices. Experiments on the interplay between grain structure from the NG regime to CG in austenitic stainless steel on osteoblast functions indicated that cell attachment, proliferation, viability, morphology and spread varied with grain size and were favorably modulated on the NG and ultrafine-grain structure. Furthermore, immunofluorescence studies demonstrated stronger vinculin signals associated with actin stress fibers in the outer regions of the cells and cellular extensions on the NG surface. The differences in the cellular response with change in grain structure are attributed to grain structure and degree of hydrophilicity. The study lays the foundation for a new branch of nanostructured materials for biomedical applications.

1. Introduction

Austenitic stainless steels and titanium alloys are two alloys widely used for biomedical applications, including devices for bone fixation, partial/total joint replacement and spring clips for the repair of large aneurysmal defects. The metal devices are used in about one-third of all replacement surgery in the United States each year, totaling a quarter of a million annually. These permanently attached artificial metallic devices, such as knee and hip joints, with an expected life span of 15 years or more, break down prematurely. Possible explanations are, but not limited to: (i) inadequate bone build-up around the implants, making them loose; (ii) metallic debris generated due to wear absorbs near the implant and is attacked by the body's immune system, leading to inflammation, death of tissue and loss of bone surrounding the implant. Thus, the continued challenge for materials in contact with the bone is the development of a material to modulate cell–substrate interactions and ensure long-term stability [1–3].

Currently, surface modification approaches such as heat treatment [2–4], powder metallurgy [5] and sintering [6,7] are being explored to introduce ultrafine-grained (UFG) structures on the surface of materials to promote osseointegration and modulation of cellular activity [6–11]. To this end, it is important to consider "both surface and bulk properties" of the implants that are in direct contact with the bone to favorably modulate cell–substrate interactions and to maintain long-term stability.

Keeping in view the long-term stability of bulk metallic implants and enhancement of cellular activity, thermo-mechanical processing and severe plastic deformation methodologies have been attempted to obtain bulk nanometer-sized grain (NG) metals [12,13]. The laboratory-scale methods explored to obtain NG structure include equal channel angular pressing [13–16], accumulative roll bonding [17–19], high-pressure torsion [20–23], multiple

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compression [24] and upsetting extrusion [25]. However, the ductility of NG materials produced by these methods is significantly low compared with that of the coarse-grained (CG) materials. A high strength–high ductility combination is an important mechanical property requirement for the long-term stability of metallic implants, which are constantly exposed to torsional and flexural stresses. The present authors recently addressed the issue of the high strength–high ductility combination in biomedical stainless steel by developing the concept of phase reversion-induced NG materials [26,27]. In this approach, deformation ($\sim$60–80%) of austenite at room temperature leads to strain-induced transformation of face-centered cubic austenite (γ) to body-centered cubic martensite (α'). Upon annealing at 700–800 °C for short durations of 10–100 s (depending on the dimensions of the sample), the martensite transforms back to austenite via a diffusional-reversion mechanism [26,27]. The approach yields a NG structure that not only results in higher strength, but also renders the material significantly ductile. The phase-reversion concept is illustrated schematically in Fig. 1.

The uniqueness of this novel concept is that it provides a great deal of latitude to obtain a range of grain sizes from the NG regime to the CG regime through change in the percentage of cold deformation and annealing temperature–time sequence. High-temperature annealing promotes grain growth with grain size in the micrometer range [26]. Thus, the approach facilitates the study of large sampling of grain size effects to develop models of cellular and molecular activity in a single material, using an identical set of parameters.

Furthermore, materials with sub-micron to nanometer-sized grains, by virtue of their high grain boundary length to grain size ratio are excellent vehicles to explain fundamentally the modulation of cellular activity and cell–substrate interactions. Thus, the concept is used here to explain the interplay between cellular activity and grain size, and constitute the objective of the proposed research. Biological response studied on the NG structure obtained via the phase-reversion concept indicated significant modulation of cell–substrate interactions and enhancement of osteoblast functions. Simultaneously, the high strength of the NG biomedical device provides the required wear resistance, in addition to thinner and reduced mass (high strength/weight ratio) for long-term stability.

The primary objective of the proposed research is to elucidate the fundamental principles and mechanisms underlying favorable modulation of cellular response in NG materials by combining materials science and engineering with cellular biology. The hypothesis is that the NG structure with a high degree of surface wettability enables cells to more readily attach and favorably modulate cell–substrate interactions. The specific aspects that the hypothesis addresses are: (a) What is the origin of enhanced osteoblasts attachment and growth on NG structures in relation to CG structures? (b) How do cellular and molecular activities on NG surfaces qualitatively and quantitatively differ from the CG structure, and how are the differences related to the surface properties? and (c) What are the fundamental mechanisms that govern cell–substrate interactions in NG materials? The present work examines in detail the effect of grain structure from the NG regime to the CG regime on MC3T3 osteoblast cell attachment and proliferation. In this respect, it is different from earlier work [27].

2. Experimental procedure

2.1. Materials, grain structure and mechanical properties

The experimental material was 316L stainless steel. Stainless steel strips were obtained from Outokumpu Stainless Oy (Torino). To develop NG structures, the stainless steel strips were cold deformed in a laboratory rolling mill using $\sim$60–80% deformation. The reversion annealing was carried out in a Gleeble-1500 thermo-simulator in the range 600–850 °C for periods of 10–100 s, to obtain different ranges of grain size in the NG regime. By altering the cold deformation and annealing sequence, various grain sizes from the NG regime to the CG regime were obtained. After annealing, the samples were force-air-cooled at a cooling rate of $\sim$200 °C s^{-1} to 400 °C, followed by convective air cooling. The annealing conditions defined here for strips are specific to the experiments described here and any generalization to application-oriented approach necessitates fine-tuning of the experimental parameters. For instance, large cross-sectional components can be annealed for a short duration to create a NG structure at the surface ($\sim$top 0.5–1 mm layer) where cellular attachment and tissue growth occurs.

The grain structure of CG was examined by light microscopy, while that of sub-micron-grained (SMG), fine-grained (FG) and NG austenitic stainless steels was examined using transmission electron microscopy (TEM). The structure of CG steels was examined after electropolishing in a solution of dilute nitric acid at a current density of $\sim$30 mA cm^{-2} and a voltage of 1.3 V DC for 2–3 min. Thin foils for examining SMG, FG and NG structures by

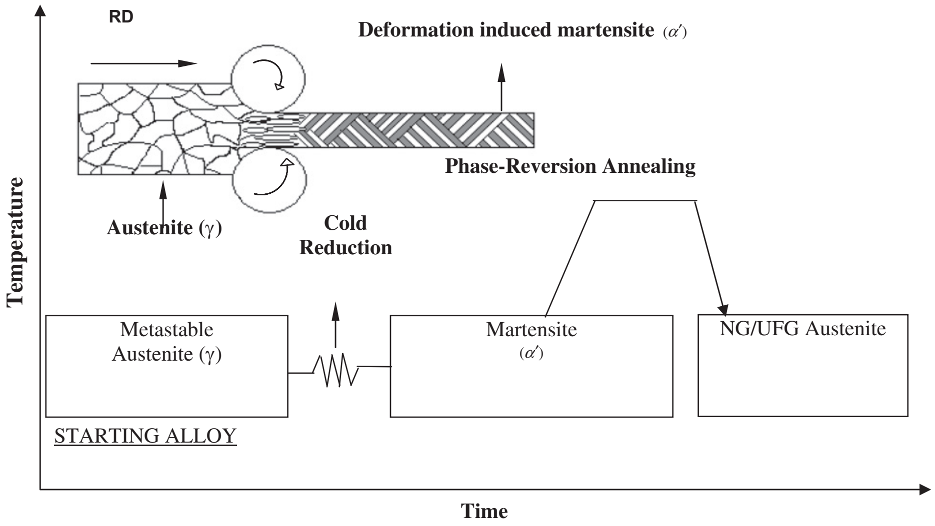

Fig. 1. Schematic representation of phase reversion concept to obtain NG structure [26,27].