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Cellular response of preosteoblasts to nanograined/ultrafine-grained structures

R.D.K. Misra [a,*], W.W. Thein-Han [a], T.C. Pesacreta [b], K.H. Hasenstein [b], M.C. Somani [c], L.P. Karjalainen [c]

[a] *Biomaterials and Biomedical Engineering Research Laboratory, Center for Structural and Functional Materials, University of Louisiana at Lafayette, P.O. Box 44130, Lafayette, LA 70504, USA*
[b] *Department of Biology, University of Louisiana at Lafayette, P.O. Box 42451, Lafayette, LA 70504, USA*
[c] *Department of Mechanical Engineering, The University of Oulu, P.O. Box 4200, 90014 Oulu, Finland*

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Abstract

Metallic materials with submicron- to nanometer-sized grains provide surfaces that are different from conventional polycrystalline materials because of the large proportion of grain boundaries with high free energy. In the study described here, the combination of cellular and molecular biology, materials science and engineering advances our understanding of cell–substrate interactions, especially the cellular activity between preosteoblasts and nanostructured metallic surfaces. Experiments on the effect of nano-/ultrafine grains have shown that cell attachment, proliferation, viability, morphology and spread are favorably modulated and significantly different from conventional coarse-grained structures. Additionally, immunofluorescence studies demonstrated stronger vinculin signals associated with actin stress fibers in the outer regions of the cells and cellular extensions on nanograined/ultrafine-grained substrate. These observations suggest enhanced cell–substrate interaction and activity. The differences in the cellular response on nanograined/ultrafine-grained and coarse-grained substrates are attributed to grain size and degree of hydrophilicity. The outcomes of the study are expected to reduce challenges to engineer bulk nanostructured materials with specific physical and surface properties for medical devices with improved cellular attachment and response. The data lay the foundation for a new branch of nanostructured materials for biomedical applications.

Keywords: Nanostructured materials; Phase reversion; Stainless steel; Cell attachment; Molecular interaction

1. Introduction

Austenitic stainless steels and titanium alloys are two widely used metallic materials for a range of biomedical applications that includes devices for bone fixation, partial/total joint replacement and spring clips for the repair of large aneurysmal defects. These materials are corrosion resistant and have the necessary mechanical strength and biocompatibility [1–3]. A potentially transformative approach to favorably modulate and improve the cellular response of biomaterials is to utilize nanograined (NG)/ultrafine-grained (UFG) materials in lieu of conventional, coarse-grained (CG) materials. Ultrafine structures may enhance cellular adhesion, stimulate metabolic activity and up-regulate protein formation [4–9]. These properties provide the motivation to study bulk nanostructured metals, with the aim to elucidate enhanced metabolic compatibility and cellular adhesion in addition to the use of thinner bioimplants with higher strength/weight ratio, especially for bone growth. The primary challenge for bone implants is the development of materials with both surface and bulk properties that improve cell–substrate interaction and ensure long-term stability of physical and mechanical properties of bioimplants. Although bulk characterization

* Corresponding author. Tel.: +1 337 482 6430; fax: +1 337 482 1220.
E-mail address: dmisra@louisiana.edu (R.D.K. Misra).

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of materials has been conducted, the significance of cell–substrate interaction in nanostructured materials has not been adequately addressed, primarily because the development of nanostructured materials is relatively new.

In determining the success of a bioimplant in vivo, surface properties are critical for substrate–tissue interaction [10,11]. The chemistry and morphology of the surface can affect the attachment and subsequent growth behavior of cells on bioimplant material and consequently the compatibility between the host tissue and implant [12,13]. Assuming that the ability of cells to adhere to a foreign surface is related to compatibility, cell adhesion is an important parameter for understanding biocompatibility [14,15]. It is safe to assume that substrate properties determine cell attachment, orientation, migration, and metabolism [15–18].

Other surface parameters that modulate cell response include hydrophilicity, roughness and texture [19]. Higher roughness promotes bone-to-implant contact [20,21] and increases removal torque forces [22–24]. The surface properties determine the degree of bioimplant integration. For osteoblasts, early colonization of the implant surface is likely to promote bone tissue repair in the peri-implant region, leading to effective osteointegration of metal implants [25,26]. The adhesion of cells to the substrate through an extracellular matrix provides signals that influence their ability to survive, proliferate and express specific developmental phenotypes [27].

The distinct and special properties of materials with submicron to nanometer-sized grains are derived from the high density of grain/interphase boundaries, which are regions of high free energy [28]. Thermo-mechanical processing (TMP) is one of the primary methods to achieve grain refinement in metals [29–32]. Grain refinement limits imposed by conventional TMP can be overcome by the application of extensive plastic deformation, which leads to the formation of submicron or ultrafine structures in metallic materials [33,34]. The laboratory scale methods that have been adopted to obtain NG/UFG materials include equal channel angular pressing [34,35], accumulative roll bonding [36–38], high-pressure torsion [39–42], multiple compression [43] and upsetting extrusion [44]. However, the ductility of the UFG materials produced by these methods is low compared to CG materials. In this context, the work presented here is of particular significance because high ductility was obtained in the NG/UFG material.

We attempt to combine fundamental aspects of materials science, engineering and biological sciences to favorably modulate the cell–substrate response between preosteoblasts and NG/UFG austenitic stainless steels. We used NG/UFG austenitic stainless steel processed by a novel procedure involving controlled phase reversion of strain-induced martensite in a cold-rolled austenitic stainless steel. Multi-pass cold deformation (~40–65%) of austenite at room temperature led to strain-induced transformation of the austenite (face-centered cubic γ) to dislocation-cell-type martensite (bcc α'), which upon annealing in the temperature range of 600–850 °C transforms to NG/UFG austenite through a diffusional reversion mechanism, depending on the temperature–time annealing sequence [45–48]. Forming experiments carried out using conventional low-rate hydraulic bulging and the high velocity electrohydraulic impulse methods for the NG/UFG stainless steels proved that the UFG steel provides a combination of high strength and good ductility. The height of the highest solid dome was 36 mm, which was achieved by 80 bar pressure in the conventional test and by 13.8 kJ energy in the high velocity test.

Given that NG/UFG structures are likely to have surface properties different from conventional CG structures, the grain size effects on cellular response of CG and NG/UFG austenitic stainless steels were investigated. The degree of cell–substrate interaction was assessed by studying the cellular activity of preosteoblast cells cultured on nanostructured austenitic steel substrate. Because proteins in the extracellular matrix of cells regulate cell–substrate interactions and influence cell adhesion, proliferation, viability and differentiation [49], fibronectin, actin and vinculin, as well as cytoskeletal organization and focal adhesion of osteoblasts, were studied. Thus, an important objective of the study was to examine how cells recognize surfaces and interact with them and each other. If the initial cell–substrate interaction is optimal, then greater quantities of extracellular matrix would be released than in less stimulated preosteoblasts. Thus, we hypothesize that a surface to which cells can more readily attach would promote cell adhesion and proliferation and lead to effective integration of implants. We examined this hypothesis by testing the differences in cellular response between NG/UFG and CG structures and their relationship to surface properties.

2. Experimental

2.1. Materials

The experimental material was commercially available 316L stainless steel. Stainless steel strips were obtained from Outokumpu Stainless Oy, Tornio and the nominal chemical composition (in wt.%) was Fe–0.017C–1.29Mn–17.3Cr–6.5Ni–0.15Mo–0.52Si. To develop NG/UFG structures, stainless steel strips were cold deformed to ~40–65% in a laboratory rolling mill using several passes (generally about 3–15% reduction per pass, depending on the available rolling loads, where the contact area determines the rolling load). The reversion annealing was carried out in a Gleeble-1500 thermomechanical simulator in the range of 600–850 °C for periods of 10–100 s to obtain NG/UFG austenitic stainless steel. For the experiments described here, the cold reduction was ~62% and annealing was carried at 800 °C for 1–10 s. The heating rate to the holding temperature was 200 °C s^{-1}. Following annealing, the steel was cooled in an air flow at rates of 200–400 °C s^{-1}.

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