Materials Letters 344 (2023) 134453

Contents lists available at ScienceDirect

Materials Letters

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

On the relationship between the grain boundary bio-physical attributes with the cells in the physiological environment

R.D.K. Misra *

Department of Metallurgical, Materials, and Biomedical Engineering, University of Texas at El Paso, 500 West University Avenue, El Paso, TX 79968, USA

ARTICLE INFO

Keywords:
Biomedical stainless steel
Nanograined
Grain boundary parameter
Cellular functions

ABSTRACT

The objective of the study presented here is to relate the grain boundary bio-physical attributes to the favorable cellular activity on the nanograined surface as compared to the coarse-grained counterpart in a biomedical stainless steel. The discovery of the contribution of grain boundary bio-physical parameter is proposed to be at least one of the important aspects from the perspective of understanding the cellular interactions, namely, cellular attachment, proliferation, and synthesis of proteins, on the nanostructured surface in relation to the conventional coarse-grained counterpart.

1. Introduction

It is important to recognize that stainless steels and titanium alloys are widely used for biomedical devices for the treatment of joints and defects [1–3]. They sometimes fail prematurely because of inadequate build-up of bone around the implants, and/or wear. In this regard, nanostructured alloys are considered promising next generation materials [2], where nanoscale surface favorably modulates cell-implant interactions in the biological environment. Simultaneously, the high strength of the nanograined metal implant is an important mechanical property requirement for long-term stability of metallic implants, provides wear resistance to withstand long term loading and is in addition to thinner and reduced mass (high strength/weight ratio). The subject of high strength-high ductility combination in biomedical stainless steel has been recently addressed via the concept of phase reversion-induced NG alloys [4–6]. The ductility of NG materials produced by other severe plastic deformation methods (e.g., ECAP) in contrast to the phase reversion process is significantly low compared to the CG materials.

Recently, cellular activity (cell adhesion, proliferation, synthesis of key proteins) on biomedical stainless steel was studied as a function of grain size from the nanograined (NG) to the coarse-grained (CG regime) using osteoblasts [6–11], including studies on nanoscale roughness [12] and nanoscale twinning [13]. The objective of the study presented here is to relate the grain boundary bio-physical attributes to the favorable cellular activity on the nanograined surface as compared to the CG microstructure in an identical stainless steel.

2. Experimental

The NG and CG grain size was obtained using phase-reversion concept developed in the author's laboratory (Fig. 1) and is described elsewhere [4–6]. In summary, the grain size is controlled by temperature–time annealing treatment of the severely cold deformed austenite. The average grain size of CG 301 LN austenitic steel was 22 ± 3 μm, while NG steel had an average grain size of 90 ± 8 nm (Fig. 1) and was determined from at least 25 micrographs using the ASTM linear-intercept method.

NG and CG samples were mechanically polished using a series of SiC papers followed by diamond suspension to almost similar surface average nanometric roughness (arithmetic mean roughness, Ra, measured from 3 μm × 3 μm scan area for CG and NG stainless steel was 1.45 ± 0.21 nm and 1.52 ± 0.29 nm, respectively).

The cell culture protocols are described in detail in previous studies [6–13]. Cell culture studies were performed using mouse pre-osteoblasts cell line MC3T3-E1 subclone 4 (USA). Briefly, cells were washed with phosphate buffered saline, incubated with 0.25% trypsin/0.53 mM EDTA for 5–7 min to detach the cells from the Petri dish, dispersed cells in trypsin/EDTA, transferred to a centrifuge tube and centrifuged at 2000 rpm for 5 min. Cell pellet obtained after centrifugation was re-suspended in culture medium and dilution carried out using culture medium to obtain the required cell concentration. Subsequently, the sterilized steel disks were placed in 24-well plate and incubated with cell suspension at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air. Polystyrene 24-well culture plates were used for control

* Corresponding author.
E-mail address: dmisra2@utep.edu.

https://doi.org/10.1016/j.matlet.2023.134453
Received 27 March 2023; Received in revised form 4 April 2023; Accepted 26 April 2023
Available online 29 April 2023

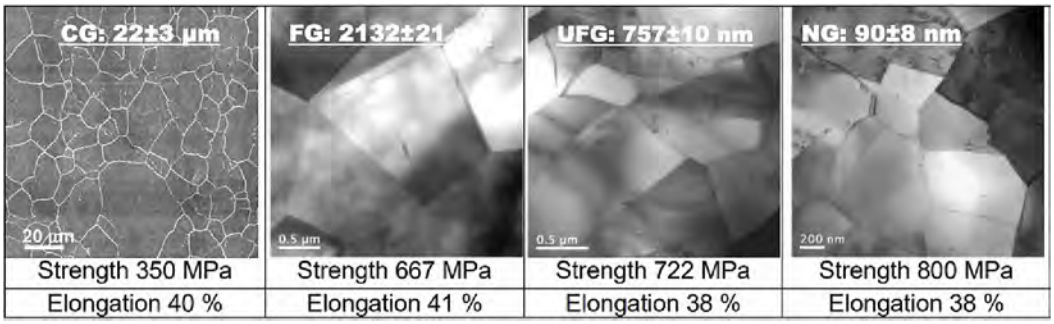

Fig. 1. Light micrograph of CG stainless steel with average grain size of 22 ± 3 μm and TEM micrographs from NG to FG. NG: nanograined; UFG: ultrafine-grained; FG: fine-grained; CG: coarse-grained.

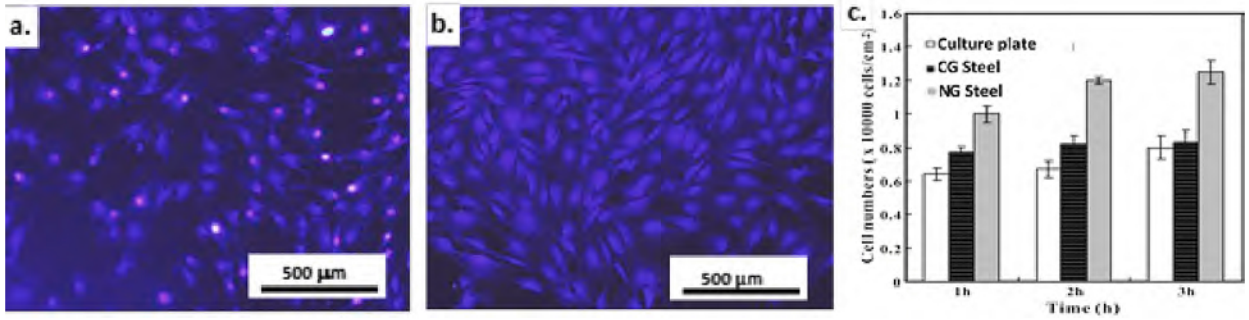

Fig. 2. Fluorescence microscopy of pre-osteoblasts stained with Hoechst 33,342 after 24 h culture on (a) CG and (b) NG substrates. Higher cell numbers with abundant extracellular matrix were observed on NG surface compared to CG substrate after 24 h. (c) The number of pre-osteoblasts grown on the NG substrate after culture time of 1–3 h exceeded the number of cells attached to the CG substrate and polystyrene culture plates (c); $p < 0.05$.

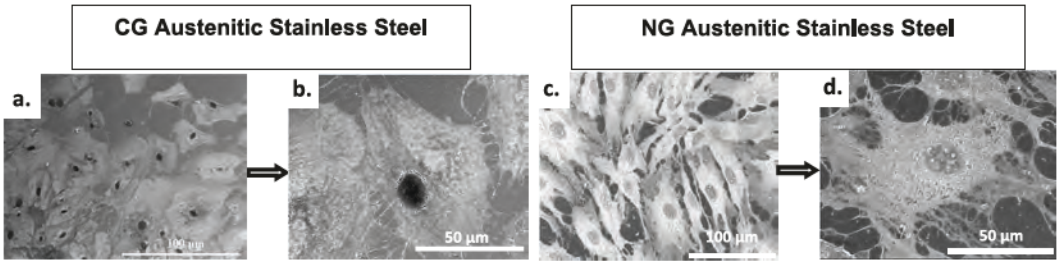

Fig. 3(i. . SEM micrographs of pre-osteoblasts cultured for 24 h on (a, b) CG and (c, d) NG stainless steels [8–10]. At low magnification (a, c) larger cell numbers are apparent on NG surface (c) than on CG surface (a). High magnification (b, d) micrographs shows a greater degree of spreading and interconnectivity on NG (d) compared to CG surface (b).

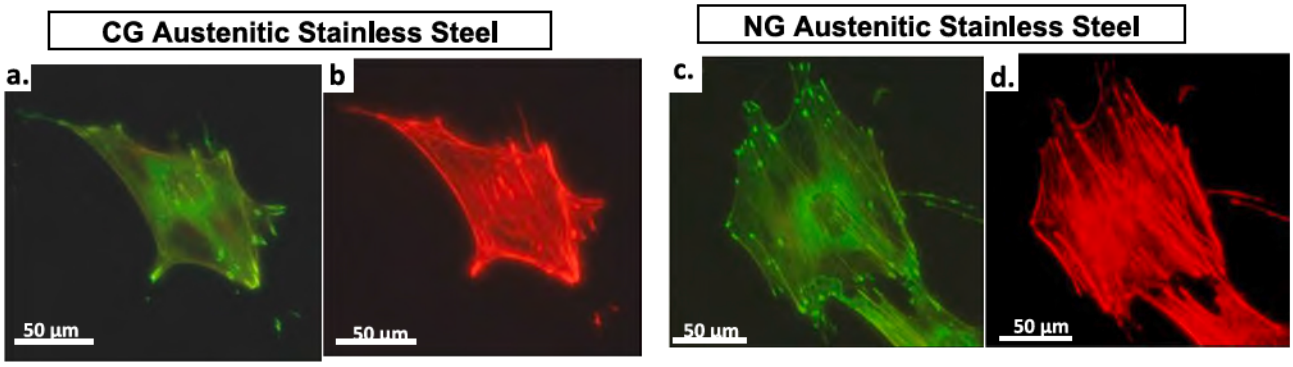

Fig. 3(ii. . Organization of vinculin focal adhesion contacts and actin stress fibers of cells after 2 days culture on (a, b) CG and (c, d) NG substrates [8–10]. Vinculin (a, c) staining shows a larger number of focal contact sites in cells grown on NG surface (c) compared to CG surface (a). The higher number of focal adhesion contacts are consistent with high number of actin stress fibers on NG steel (d) than on CG steel (b).

Table 1
Relationship between grain boundary parameters bio-physical parameters and average grain boundary length/cell for NG and CG surfaces.

	Average area of the surface covered by the cells (μm^2)	Average total grain boundary length/surface area ($\mu m/mm^2$)	Average grain boundary length/ cell (μm)
NG	1252.5	15.5	19,414
CG	517.8	0.14	71

experiments.

3. Results and discussion

The attachment of cells (blue spots in Fig. 2a, b) on NG and CG indicated good cytocompatibility with the pre-osteoblasts, but with a difference. The cells had greater attachment to the NG surface compared to the CG surface and the greater attachment was visible within one hour of cell culture. This suggested that the cell attachment to the surface was influenced by the grain size and was not a function of cell development or adaptation over time.

The cell spreading pattern (Fig. 3 (i) exhibited a different morphology on NG and CG surfaces. On CG surface, the cell morphology after 24 h culture was compact with less well-defined periphery (Fig. 3 (i) a, b), while the NG surface induced a significant change in the morphology of cells (Fig. 3(i) 3c, d), such that the cells spread uniformly with less well-defined boundaries and were elongated. On the NG surface, cells appeared flat and had star-like morphology with numerous cytoplasmic extensions, overlapping each other and forming cytoplasmic bridges (Fig. 3 (i) c, d), implying significant degree of cell-substrate interaction. Thus, the extent of cell spreading was remarkably greater on the NG surface compared to the CG surface. Examining individual proteins, actin, and vinculin, which are linked to cellular activity indicated that the NG surface produced favorable results. The expression of vinculin and actin that form focal contacts and stress fibers, showed higher expression level at the edges and well-defined stress fibers on NG steel than on the CG steel (Fig. 3i).

The summary of the above studies (Figs. 2 and 3) provided the foundation to consider the relationship between grain boundary bio-physical parameters. Analysis of at least 10 light (for CG) and transmission electron (for NG) micrographs indicated significant difference in the average grain boundary length/surface area and was ~15.5 $\mu m/\mu m^2$ and ~0.14 $\mu m/\mu m^2$ for NG and CG microstructures, respectively. Also, the average distance between grain boundaries and the dimensions of cells was significantly different for the NG and CG microstructures. Considering the representative average width of osteoblasts of 5–20 μm, as seen in Figs. 2 and 3, on the NG surface, an attached cell covered ~25–75 grains by width and ~720 grains by length. In striking contrast, a similar average size of cell on the CG surface covered ~1–3 grains by width and ~15–24 grains by length. Thus, the NG surface had significantly greater number of grain boundaries to surface-attached cells. Based on this analysis, a biophysical parameter, average grain boundary length/cell was defined that considered the physical aspect of the surface, namely, average grain boundary length/area with the cell characteristic, i.e., average area of the surface covered by the cells. The biophysical parameter, average grain boundary length/cell was defined as the ratio of the average grain boundary length/area to the average area of the surface covered by the cells [14]. Table 1 presents interesting data and positive relationship of cell attachment with the biophysical parameter, viz., grain boundary length/surface attached cells, where more than 20 times the average length of grain boundaries/cell was occupied by the cells on the NG surface as compared to the CG counterpart such that the intercept length of ~40–60 nm of the NG surface is similar to the average separation distance of cell adhesion contact or endothelial cells (~40 nm) [15].

Thus, we envisage at this time that the high density of grain boundaries in the NG microstructure (versus CG microstructure) provided greater opportunities for cell-surface interactions by impacting cell signaling and mechano-transduction pathways. This aspect is related to the physical characteristics of the grain boundaries, which favorably promotes interaction of cells with grain boundaries. The study suggests that the cells globally interact with the NG surface in a manner similar to the CG surface. Upon attachment to the surface, the cell explores the surrounding biological environment and migrates via lamellipodia and filopodia, such that their ends establish focal adhesion. This potentially occur for subsequent migration and communication between cells leading to proliferation and mineralization of the extracellular matrix (ECM) by the differentiating osteoblasts.

The results summarized above suggest a clear impact of nanoscale feature when the cells interact with the NG surface. This commences with greater attachment of cells to the nanoscale-surface, followed by proliferation, and synthesis of proteins. Thus, the increased cellular activity on the NG surface is related to the relative influence of grain boundary bio-physical attributes (Table 1) and associated mechanisms. Studies are in progress to elucidate a molecular physiological mechanism that links the physical and chemical attributes of the NG surface.

4. Conclusions

The high density of grain boundaries in the NG microstructure provided greater opportunities for cell-substrate interactions. The cells globally interact with NG surface in a manner to the CG surface. This commences with greater attachment of cells to the nanoscale-surface, followed by proliferation and synthesis of proteins. Grain boundary bio-physical parameter is at least one of the attributes that governs the increased cellular activity on the NG surface.

CRediT authorship contribution statement

R.D.K. Misra: Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgements

R.D.K. Misra is grateful to National Science Foundation for financial support through grant # CBET 2224942 (Program Manager: Dr. Nora F. Savage).

References

[1] V. Borsari, G. Giavaresi, et al., J. Biomed. Mater. Res. B 75B (2005) 359.
[2] G. Balasundaram, T. Webster, J. Biomed. Mater. Res. A 80 (2007) 602.
[3] G. Willman, Adv. Eng. Matls 1 (1999) 95.
[4] R.D.K. Misra, Y.S.Y. Injeti, et al., Scientific Rep. – Nat. 8 (7908) (2018) 1–13.
[5] R.D.K. Misra, V.S.A. Challa, et al., Acta Mater. 84 (2015) 339.
[6] R.D.K. Misra, S. Nayak, et al., Metal. Matls Trans. A 41 A (2015) 3.
[7] R.D.K. Misra, W.W. Thein-Han, et al., Adv. Mater. 21 (2009) 1280.
[8] R.D.K. Misra, W.W. Thein-Han, et al., Acta Biomater. 5 (2009) 1455.
[9] R.D.K. Misra, W.W. Thein-Han, et al., Acta Biomater. 6 (2010) 3339.
[10] R.D.K. Misra, W.W. Thein-Han, et al., Acta Biomater. 6 (2010) 2826.
[11] P.K.C. Venkatsurya, et al., Acta Biomater. 6 (2010) 2826.
[12] R.D.K. Misra, et al., Mater. Technol. 37 (2022) 437.
[13] K.C. Nune, et al., J. Mech. Behav. Biomater. 88 (2018) 185.
[14] T.C. Lowe, R.A. Reiss, et al., Mater. Res. Lett. 8 (2020) 239.
[15] G. Le Saux, et al., Biophys. J. 101 (2011) 76.