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Published in:
ACS Biomaterials Science & Engineering

DOI:
10.1021/acsbiomaterials.0c01034

Publication date:
2021

ACS Biomaterials SCIENCE & ENGINEERING

pubs.acs.org/journal/abseba Article

Texture-Governed Cell Response to Severely Deformed Titanium

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Cite This: *ACS Biomater. Sci. Eng.* 2021, 7, 114–121 Read Online

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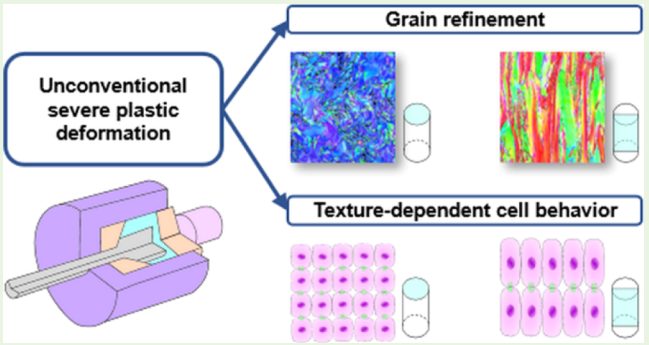
ABSTRACT: The phenomenon of superior biological behavior observed in titanium processed by an unconventional severe plastic deformation method, that is, hydrostatic extrusion, has been described within the present study. In doing so, specimens varying significantly in the crystallographic orientation of grains, yet exhibiting comparable grain refinement, were meticulously investigated. The aim was to find the clear origin of enhanced biocompatibility of titanium-based materials, having microstructures scaled down to the submicron range. Texture, microstructure, and surface characteristics, that is, wettability, roughness, and chemical composition, were examined as well as protein adsorption tests and cell response studies were carried out. It has been concluded that, irrespective of surface properties and mean grain size, the $(10\bar{1}0)$ crystallographic plane favors endothelial cell attachment on the surface of the severely deformed titanium. Interestingly, an enhanced albumin, fibronectin, and serum adsorption as well as clearly directional growth of the cells with preferentially oriented cell nuclei have been observed on the surfaces having (0001) planes exposed predominantly. Overall, the biological response of titanium fabricated by severe plastic deformation techniques is derived from the synergistic effect of surface irregularities, being the effect of refined microstructures, surface chemistry, and crystallographic orientation of grains rather than grain refinement itself.



KEYWORDS: *severe plastic deformation (SPD), electron backscattering diffraction (EBSD), titanium, texture, cell response*

1. INTRODUCTION

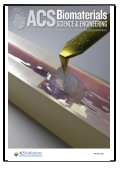
It is generally acknowledged that the fine-grained, ultrafine-grained, and nanocrystalline titanium-based materials manufactured by means of severe plastic deformation (SPD) approaches are characterized by a set of remarkable mechanical and functional properties.[1] Moreover, the biological performance of the SPD-produced titanium has also proven to be enhanced as promoted adsorption of proteins along with more efficient attachment, proliferation, and differentiation of a variety of cell types, including osteoblasts, fibroblasts, and keratinocytes, has been observed.[2−6] The phenomenon has been under discussion for more than a decade but is yet to be completely fathomed.

Commonly, the origin of superior biological behavior of the SPD-fabricated materials is related to the substantial differences in surface topography and its physicochemical properties, stemming from microstructural changes during the introduction of heavy strains into the volume of processed specimens.[2,6] So far, grain refinement, improved hydrophilicity and roughness, an augmented fraction of high-angle grain boundaries (HAGBs), as well as surface irregularities, in the form of nanodefects or nanogrooves, have been claimed to affect the cytocompatibility of SPD-processed titanium.[2,3,7−12]

Grain refinement, commonly practiced as a way of strengthening materials, has been somehow naturally linked to the enhancement of biological properties of the SPD materials. However, crystallographic orientation of grains in a deformed sample has been currently addressed in the biological assessment of metallic substrates,[13−18] because it is well known that a great deal of properties, such as ductility, corrosion resistance, or magnetic permeability, are influenced by texture. Nevertheless, a clear evidence-based study proving the arrangement of cells with respect to the orientation of grains has not been reported yet.

Bearing in mind the existing gap in understanding texture−cell response relationship, the present study highlights the impact of crystallographic orientation of grains in titanium manufactured by hydrostatic extrusion (HE) on its interaction with biological components. It needs to be emphasized that

Received: July 11, 2020
Accepted: December 10, 2020
Published: December 21, 2020



https://dx.doi.org/10.1021/acsbiomaterials.0c01034
ACS Biomater. Sci. Eng. 2021, 7, 114−121

modulation of cell response for both coarse-grained and ultrafine-grained titanium actually refers to the properties of a thin, well-adhered, and thermodynamically stable layer that is spontaneously formed on the surfaces of easily passivated metals as soon as being exposed to the environments enriched with oxygen.[19]

2. MATERIALS AND METHODS

2.1. Material Development. The material investigated was commercially pure titanium (grade 2) subjected to four-step HE, being the process of an unconventional severe plastic deformation. The technique, whose principles could be found elsewhere,[20] was utilized for the sake of improvement in the mechanical properties of unalloyed titanium. HE has been perfected to such a degree that it enables the manufacture of pure titanium-based materials outperforming the Ti−6Al−4V alloy in terms of strength.[20,21] Prior to the actual deformation, the samples were machined, then annealed at 700 °C for 2 h, so the material became homogenized and softened. The billet, having 25 mm of a diameter in its initial condition, was processed to the final rod with a diameter of 7.41 mm. Deformation was maintained without any post-annealing treatment; instead, the material obtained was simply air-cooled. Between the consecutive extrusions, water quenching was realized to minimize the adiabatic heating effects.

2.2. Microstructure and Texture Examination. All of the investigated samples were progressively abraded with a set of silicon carbide foils, ranging from 100 to 4000 grit, electro-etched using the alcohol-based solution, flushed with isopropanol, and dried with the stream of hot air. In order to execute the microstructural and textural analyses, electron backscatter diffraction (EBSD, FEI Quanta 3D FEG SEM combined with EDAX/OIM/TSL/EBSD facility), transmission electron microscopy (ECNAI SuperTWIN G2 FEG200 kV equipped with HAADF/STEM/EDAX attachments), and X-ray powder diffraction (Bruker D8 diffractometer, using filtered Co Kα radiation at a wavelength of 0.1789 nm) were applied.

2.3. Surface Properties Examination. The surfaces of the investigated materials were analyzed with regard to wettability, roughness, topography, and chemical composition. A static contact angle was assessed by the sessile drop method by using a DSA 100 Krüss contact angle goniometer equipped with a video capture attachment. The atomic force microscopy measurements were performed with the use of an Innova commercial instrument. Surface chemistry was examined by using a PHI VersaProbeII Scanning XPS system employing monochromatic Al Kα (1486.6 eV) X-rays.

2.4. Biological Properties Examination. So as to eliminate the impact of surface roughness and wettability on protein adsorption and cellular response, mirror-polished specimens were prepared in a manner guaranteeing near-identical topographies. In doing so, the aforementioned standard metallographic procedures were employed. Bovine serum albumin (BSA), bovine fibronectin protein (BFP), and fetal bovine serum (FBS) adsorption to the HE-processed titanium surfaces were estimated by a quantity assay. The protein concentration within the eluted samples was evaluated using the Qubit Protein Assay Kit and a Qubit fluorometer according to the manufacturer's instruction. Cellular response studies were performed with human umbilical vein endothelial cells (HUVECs). To the authors' knowledge, the behavior of this particular cell line on the surfaces of the SPD-produced titanium has not been the subject of any analysis yet. Cell−substrate interactions were determined based on the morphology and proliferation in 72 h of the cell culture. Prior to the microstructural analyses, the samples were stained to visualize F-actin fibers of cytoskeleton and cell nuclei. Afterward, a confocal laser scanning microscopy (CLSM) Exciter 5 AxioImager was utilized. In addition, the Image Processing Toolbox module in Matlab was implemented in order to trace the regularity of nuclei orientation.

3. RESULTS

The microstructure of the HE-processed titanium was investigated at first, and the resultant orientation imaging microscopy (OIM) maps, obtained by using the EBSD method, are shown in Figure 1. Additionally, transmission electron microscopy (TEM) technique was implemented so as to gain a better insight into the material's microstructure. Both of the orthogonal cross sections were characterized as the SPD-fabricated materials tend to manifest the anisotropy of microstructures and properties.[19] For convenience, Ti−T and Ti−L refer to the transverse and the longitudinal cross section of the examined samples, respectively. Regardless of the cross-section studied, the microstructure of the HE-processed

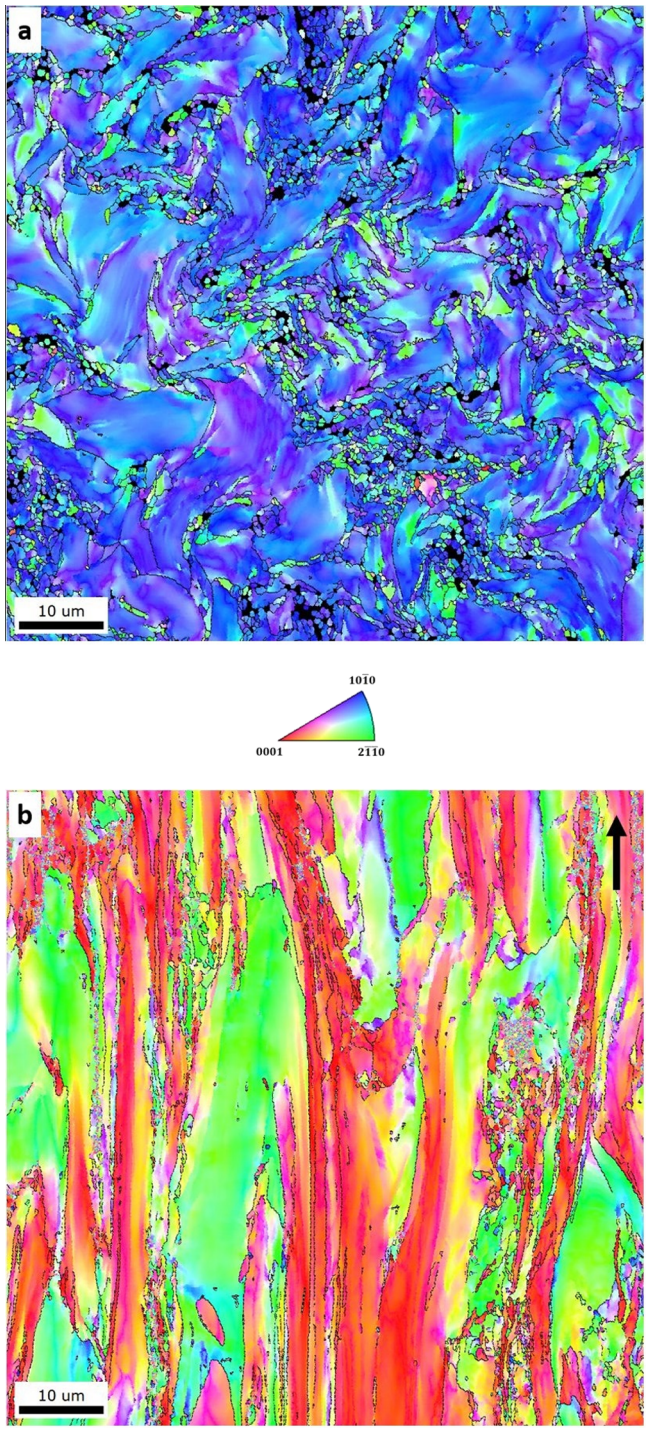


Figure 1. OIM maps taken from (a) Ti−T and (b) Ti−L.

https://dx.doi.org/10.1021/acsbiomaterials.0c01034
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Table 1. Microstructural Characteristics of the Investigated Cross Sections

cross section	ultrafine grains share (%)	mean ultrafine grain size (μm)	GOS (°)	LAGB density ($\mu m/\mu m^2$)	HAGB density ($\mu m/\mu m^2$)
Ti−T	19.4	0.66 ± 0.17	15.8 ± 14.6	0.34	1.11
Ti−L	11.5	0.61 ± 0.20	17.8 ± 10.6	0.34	0.91

titanium was bimodal with grains exhibiting high local misorientation and considerable share of structural defects (see some characteristics listed in Table 1). The microstructure analyzed on the Ti−T was composed of wavy, refined grains with 19.4% fraction of the ultrafine ones. On the other hand, grains elongated in the extrusion direction (marked by a black arrow) were clearly observed while investigating the Ti−L microstructure. The share of ultrafine grains determined for the Ti−L specimen was approximately 11.5%.

As clearly seen from the TEM images, illustrated in Figure 2, hydrostatically extruded titanium is characterized by a non-

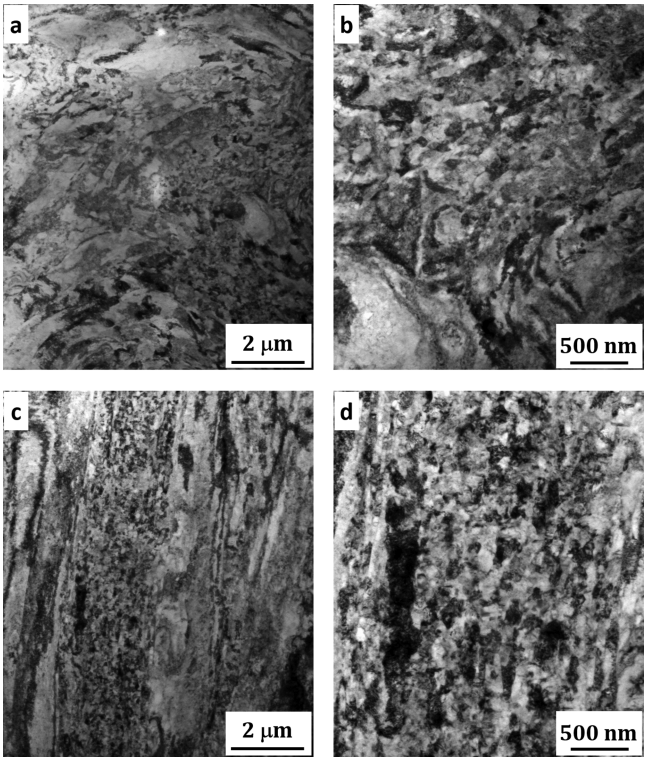

Figure 2. TEM images taken from (a,b) Ti−T and (c,d) Ti−L.

homogeneous microstructure, in which ultrafine grains and close-to-equiaxed nanograins are accompanied by coarse grains, irrespective of the cross section analyzed. Large grains contain high-density dislocations and a pronounced fraction of subgrains. Moreover, boundaries between single grains are locally hard to distinguish, which indicates a substantial fraction of low-angle grain boundaries, typically consisting of dislocation arrays, in the volume of a material.[21]

Overall, from the grain refinement point of view, not only did both the microstructures demonstrate similar features (e.g., share of defects) but also the difference in the calculated values of the ultrafine grain size was not of great importance. What the examined samples differs fairly is the crystallographic orientation of grains. Inverse pole figures (IPFs) determined by the EBSD technique for the analyzed substrates are depicted in Figure 3.

By means of plastic deformation through HE, texture of materials is modified from weak to $(10\bar{1}0)$ fiber.[21] In the case of

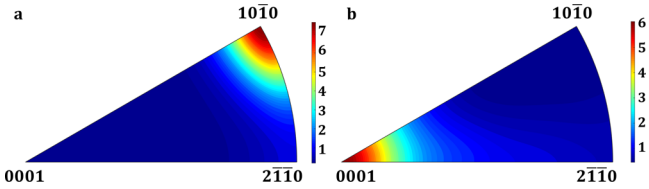

Figure 3. {001} IPFs taken from (a) Ti−T and (b) Ti−L.

HE-processed titanium, the texture is axial owing to the fact that subjecting the material to consecutive axially symmetric HE stages strengthens the fiber component. On the Ti−T, the grains having the $(10\bar{1}0)$ plane parallel to the surface are almost exclusively observed, whereas on the Ti−L, the grains are distributed between (0001) and $(2\bar{1}\bar{1}0)$ crystallographic planes (as can be seen in Figure 1b).

Because of the fact that the excellent physicochemical properties of a material's surface reflect on the biological response,[12,15] roughness as well as wettability evaluation was carried out. The corresponding results are presented in Table 2 and Figure S1. No significant difference in surface nanoroughness was reported ($p < 0.05$). Nevertheless, the specimens varied notably in terms of surface topography. Characteristics such as peak density and mean spacing revealed more increased surface area of the Ti−T specimens than that of the Ti−L ones. High values of peak density are entangled with lower values of mean spacing, reflecting defect-enriched surfaces. In addition, wettability, determined for the HE-processed titanium from the water contact angle measurements, fell within the range of 75.5−78.5°, indicating a clearly hydrophobic nature of the surface. The discrepancies between the investigated cross sections were statistically insignificant ($p < 0.05$). It is well known that substrates exhibiting a hydrophilic character tend to adsorb proteins more readily.[16] Interestingly, within the present study, the adsorption of BSA, BFP, and FBS was reported to be specific for the HE-processed titanium, as can be seen in Figure 4. The Ti−L substrates experienced superior adsorption of the analyzed proteins with comparison to the Ti−T ones (statistically significant differences, $p < 0.05$). However, both of the examined surfaces manifested hydrophobic nature; therefore, the effect of wettability on protein adsorption could be, beyond any doubt, neglected.

The chemical composition of the titanium passive layers gathered from the XPS spectra are shown in Figure S2 and Table 3. Titanium, oxygen, carbon, and calcium make up for the main elements detected. Because none of the carbon and calcium peaks were recorded at higher energy binding regions, it is believed that their presence on the surfaces could be assigned to contamination compounds, for example, CaO and $CaCO_3$. Regardless of the analyzed sample, Ti 2p spectra displayed two major peaks, that is, at 465 and 458.1 eV binding energies, originating from the Ti $2p_{1/2}$ and Ti $2p_{3/2}$ states of stoichiometric TiO_2, respectively. The Ti−T substrates were thinner and characterized by a more diversified surface composition as peaks, indicating the existence of metallic Ti, Ti(II) oxidation state in TiO and Ti(III) in Ti_2O_3 were more pronounced in comparison to the Ti−L specimens. For both the

https://dx.doi.org/10.1021/acsbiomaterials.0c01034
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Table 2. Surface Characteristics of the Investigated Cross Sections

cross section	contact angle (°)	roughness (nm)	surface defects density ($1/\mu m^2$)	x-peak density ($1/\mu m$)	y-peak density ($1/\mu m$)	x-meanspacing (μm)	y-mean spacing (μm)
Ti−T	76.9 ± 1.4	1.51 ± 0.43	7.11	2.13 ± 0.09	3.34 ± 0.06	0.47 ± 0.02	0.30 ± 0.01
Ti−L	77.8 ± 0.7	1.84 ± 0.18	1.49	1.20 ± 0.07	1.24 ± 0.07	1.18 ± 0.08	2.07 ± 0.41

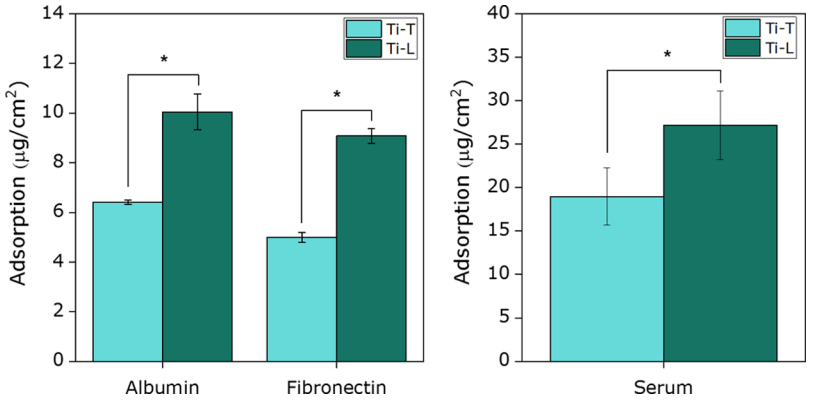

Figure 4. Protein adsorption on the HE-processed Ti. The asterisk sign denotes statistical significance between the probes ($p < 0.05$).

Table 3. Relative Percentages of Ti and O Detected on the Examined Surfaces

cross section	$[Ti^{Me}]$	$[Ti^{2+}]$	$[Ti^{3+}]$	$[Ti^{4+}]$	$[Ti^{Me}]/[Ti^{4+}]$	$[O_2^-]$	$[OH^-]$	$[OH^-]/[O_2^-]$
Ti−T	0.1	0.06	0.14	0.70	0.14	0.74	0.26	0.35
Ti−L	0.03	0.02	0.13	0.82	0.04	0.68	0.32	0.47

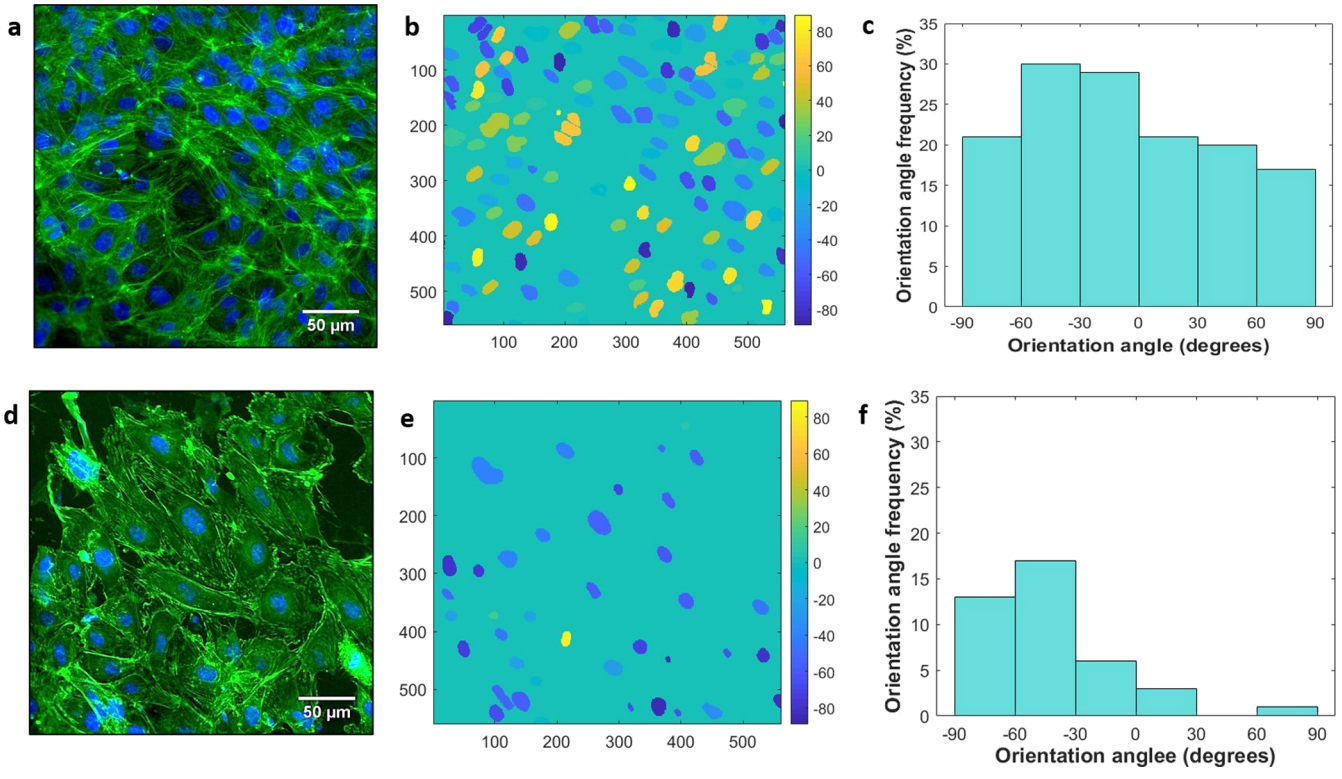

Figure 5. (a) CLSM image, (b) nuclei orientation map, (c) orientation angle histogram for the Ti−T; (d) CLSM image, (e) nuclei orientation map, and (f) orientation angle histogram.

samples, the deconvoluted O 1s spectra revealed a slight hump at 532.5 eV, related to the surface-adsorbed oxygen, associated with the presence of hydroxyl groups on the surface. However, the relative percentage of the OH^- group was greater for the Ti−L substrates.

CLSM observations of the endothelial cells incubated on the severely deformed titanium for 72 h revealed considerable differences in cell density as well as cell spreading patterns. Proliferation of the cells on the Ti−Ti and Ti−L surfaces is depicted in Figure 5a,d, respectively. HUVECs were able to

https://dx.doi.org/10.1021/acsbiomaterials.0c01034
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cover the entire surface with a confluent monolayer, regardless of the cross section analyzed. In the case of Ti−T substrates, a couple of layers were formed as the surfaces experienced more efficient proliferation in comparison with the Ti−L substrates. The vast majority of the cells grown on the Ti−T surfaces exhibited phenotypic morphology, including flattened and polygonal shapes. Moreover, a great deal of cytoplasmic extensions has already been formed at this stage of incubation. Contrarily, on the Ti−L surfaces, HUVECs, having clearly elongated shapes, were spread out in a preferential manner and nuclei also manifested directional alignment. In addition, the active processes of filopodia formation were noticeable.

Owing to the fact that discrimination and meticulous quantitative analyses of the single cells from CLSM images could be burdened with errors, orientation of single cell nuclei was quantified by implementing image analysis approach. Nuclei orientation angle maps along with the orientation angle histograms estimated for both of the studied cross sections are presented in Figure 5. Although it is apparent that the orientation of the HUVECs nuclei observed on the surface of Ti−T was random, the Ti−L surface was covered with cells having nuclei oriented in a privileged manner. Graphical analyses and microstructural characterization of the Ti−L surfaces implied that the endothelial cells were positioned parallelly to the extrusion direction, overlapping with the $[10\bar{1}0]$ crystallographic direction of the elongated grains. This observation indicates the impact of crystallographic texture of the SPD-fabricated titanium on cellular response.

4. DISCUSSION

Microstructure of the SPD-fabricated substrates appears not to be the prime factor responsible for the biological properties of severely deformed titanium, although it has been usually claimed to be.[2,3,6,22,23] Nonetheless, the microstructural changes that a material undergoes while being subjected to the SPD techniques are striking and thereby worth underlining. The HE-processed titanium is characterized by the very high values of GOS, a parameter used to describe the intragranular misorientation. It indicates an evident subgrain structure along with a considerable dislocation density accumulated within the analyzed microstructures. What is especially relevant, and needs to be stressed out, is that the increased fraction of grain boundaries, serving as optimal sites for the processes of focal adhesion,[12,17] could substantiate enhanced cell response of a material as it is known that well-developed, high-angle grain boundaries tend to affect, for example, mechanical properties and corrosion resistance.[24]

It is noteworthy to point out that grain refinement realized through the SPD methods and accompanied by generation, rearrangement, and annihilation of dislocations results typically in substantial changes of the nano-scaled topography as well as development of highly deformed subsurfaces.[21] Herein, electropolishing was implemented as a surface modification process in order to form strain- and defect-free subsurfaces as it has been already declared that any manufacturing related imperfections could mightily alter protein adsorption and subsequent cell−substrate interactions.[25] A primary advantage of the ultrafine-grained and nanostructured materials over the coarse-grained ones is that they offer a notably larger specific surface area reflected in a tremendous number of nano-scaled features spanning on the surface. Hence, a variety of defects, that is, nanogrooves or nanopeaks, acting as adsorption and adhesion sites, facilitate proteins to get adsorbed and cells to adhere, respectively. In addition, defect-enriched surfaces tend to modulate integrins to identify all of the proteins that have already been absorbed on the surface.[26] Generally, nanoscale features imitate the architecture of the extracellular matrix, viewed as the microenvironment interacting with cells. Their biological significance is profound as they are able to influence the type, quantity, and conformation of adsorbed proteins. Moreover, integrin signaling and signaling pathways, both controlling cell activity, are also affected by surface defects.[27]

Protein adsorption, cell adhesion, and proliferation processes are governed by the physicochemical properties of a substrate, primarily hydrophilicity/wettability and surface roughness.[15,28] Adsorption of proteins on various deformed surfaces may be attributed to additional factors such as electrostatic interactions between protein molecules and surface as well as the type of proteins and their conformation.[29] Apart from wettability and roughness, surface chemistry could also have a vital role in governing the biological properties of a material. It has been stated that physicochemical properties of the surface get altered when a material is subjected to various, complex plastic deformations.[30] Titania layer formed on the HE-manufactured Ti has shown to be slightly thicker and covered with a greater amount of hydroxyl groups when compared to the unprocessed substrates.[2] In addition, hydrostatically extruded titanium demonstrates anisotropy of corrosion resistance in synthetic saliva.[31] Overall, nanostructuring has shown to result in the presence of more compact and stable passive films on the surfaces of the SPD-processed materials because the improved diffusion of elements takes place.[4]

Interestingly, while analyzing the ECAP-produced titanium samples, an unequal concentration of functional groups was found on the surface, considering varied crystallographic orientation of grains in the examined substrates. Densely packed basal planes have proven to favor the presence of hydroxyl groups and, consequently, drive the formation of Ti−OH bonds.[14] Such a finding is consonant with the investigations revealed herein. Higher amount of OH^- groups was detected for the Ti−L surfaces, having mainly the (0001) planes exposed. Commonly, it has been noted that the increased level of OH^- moieties on the surface of a metallic sample regulates protein adsorption and cell−substrate interactions.[32]

Within the present study, the characterized substrates of clearly hydrophobic nature experienced similar nanoroughness, yet differed greatly with regard to high-angle grain boundary density and surface characteristics in the form of peak density and mean spacing. Nevertheless, the Ti−T specimens, manifesting an increased share of surface nanofeatures, exhibited lower protein adsorption. Such unusual behavior might only be a result of different protein conformations as well as various surface chemistries, originating from clear differences in crystallographic orientation of grains. However, not only the presence of functional groups but also their distribution ought to be taken into considerations. What deserves mentioning is that by altering the density of functional groups on the surface, it is possible to tune protein adsorption mechanisms. Near-to-zero adsorption or the formation of a monolayer may be accomplished.[33]

Overall, protein adsorption is followed by cell adhesion, which is a complex, multistage mechanism, encompassing surface recognition by cells, development of focal contacts, and cell spreading.[18] During the so-called initial cell events, cell adhesion is a process of paramount importance because of the fact that its overall efficiency determines the late cell−biomaterial interactions. Proliferation, migration, and differentiation are

https://dx.doi.org/10.1021/acsbiomaterials.0c01034
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regulated by adhesion of cells to the substrate.[16] Factors affecting an initial cell attachment involve electrostatic interactions as well as van der Waals interactions between the cells and the surface, ionic bonding, and hydrophilicity. In the present study, both of the investigated surfaces, varying in crystallographic orientation of grains, were covered with a confluent monolayer. Nevertheless, the proliferation of endothelial cells on the SPD-produced titanium surfaces has demonstrated, depending on the cross section analyzed, varied patterns. Therefore, it may be concluded that titanium manufactured by the HE technique manifests anisotropy of not only the microstructure but also the material−cell interactions, viewed as surface-dependent phenomena.

The CLSM images unveiled the increased cell spreading throughout the surfaces dominated with the $(10\bar{1}0)$ crystallographic planes. Furthermore, clearly directional distribution of HUVECs was observed while studying cell response on the surfaces, having mostly (0001) planes exposed. The shape of cells adhered to the Ti−L substrates resembled the shape of microscale grains. However, the elongated shape of grains results from the manufacturing process and it does not directly affect biological processes at the interfaces between the titania layer and biomolecules/cells. On the other side, the shape of grains influences surface topography. Basically, different endothelial cell response is the result of the conformational changes in the structure of the extracellular matrix components, including collagen, fibronectin, and laminin.[16] These proteins tend to regulate the specific cell behavior and its activity with the surrounding environment. They are also present in the culture media supplemented with serum, thus modulating the processes of cell signaling, nuclear organization, and formation of cytoskeletal networks by binding cell adhesion receptors under *in vitro* conditions.[34] Bearing in mind such crucial functions of the serum molecules, the role of protein composition and conformation on cell adhesion is evident and foremost. Herein, more pronounced activity of HUVECs on the Ti−T substrates could be linked to the texture-related surface defects that may adjust the binding sites of proteins and, as a consequence, affect their conformation.

Recently, it has been proven that the prismatic planes, exhibiting higher surface free energy (1049 erg/cm^2) than the basal planes (988 erg/cm^2), are more prone to etching; therefore, roughened, hydrophilic, and biologically active surfaces are produced.[15] A similar effect was discovered, while examining titanium sheets and rods produced by extrusion.[13] More efficient cell attachment and proliferation was observed for substrates with the preferred orientation of $(10\bar{1}0)$ as compared with the (0002) orientation.[13] Although in the present study, both the examined surfaces were hydrophobic, a slightly thinner passive oxide film for the Ti−T samples was noted based on the XPS spectra. It could also substantiate susceptibility of nonbasal planes to etching and resulting biological properties.

Different protein adsorption and clearly directional cellular response should be attributed to various surface chemical composition, the presence of the nanostructured features, such as arrays of ultrafine grains, nanogrooves, nanopeaks on the surface, and, to a greater extent, texture of the processed materials. The crystallographic orientation of grains has shown to strongly impact protein adsorption and HUVECs adhesion as well as proliferation. Diverse cell behavior of the HE-processed titanium substrates should be linked to specific protein adsorption on different crystallographic planes and, as a consequence, conformational changes of macromolecules adsorbed on the surfaces. Having a prismatic plane directly exposed to the surface might be beneficial to biomaterial−cell interactions at the initial stage as cellular response has displayed to be mediated more effectively by the prismatic planes in comparison to the basal ones. This claim, however, needs wealth of further investigations. The phenomenon of the interplay between proteins and substrates remains poorly understood,[25] yet it may be stated that the biomaterial−cell interactions, reported herein, are regulated by varied protein affinity to different crystallographic planes exposed to the surface.

5. CONCLUSIONS

Pure titanium subjected to HE has been investigated within the present study with the aim to find the origin of improved biological properties in the severely deformed titanium-based materials. Owing to their anisotropy, different cross sections of the obtained rods were meticulously tested. By intentionally eliminating the impact of surface roughness and wettability on protein adsorption and subsequent cell−substrate interactions, it has been shown that endothelial cell behavior tends to be diversified and clearly modulated by different crystallographic orientations of grains, but not their size. In comparison to the basal planes, substrates having mostly the prismatic planes distributed parallelly to their surface proved to foster the attachment and proliferation of cells. However, a clearly directional distribution of HUVECs was observed for the samples with the basal planes exposed. Therefore, a main conclusion to be drawn is that the biological performance of titanium processed by the severe plastic deformation methods is directly linked to the differences in crystallographic orientation of grains, surface chemical composition, and surface irregularities as they were the only significantly varying characteristics among the examined substrates. The dependence of crystallographic orientation of grains on the late cell events, such as differentiation, should be addressed in the future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsbiomaterials.0c01034.

AFM topographies of the investigated substrates along with the XPS deconvoluted spectra of Ti 2p and O 1s (PDF)

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https://dx.doi.org/10.1021/acsbiomaterials.0c01034
ACS Biomater. Sci. Eng. 2021, 7, 114−121

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

D.W. has been partly supported by the EU Project POWR.03.02.00−00-I004/16, while JK acknowledges the financial support from the Polish National Science Centre under grant number: UMO-2015/19/B/ST8/01079.

■ ABBREVIATIONS

BSA, bovine serum albumin; BFP, bovine fibronectin protein; CLSM, confocal laser scanning microscopy; EBSD, electron backscatter diffraction; FBS, fetal bovine serum; GOS, grain orientation spread; HAGB, high-angle grain boundary; HE, hydrostatic extrusion; HUVEC, human umbilical vein endothelial cell; OIM, orientation imaging microscopy; SPD, severe plastic deformation; TEM, transmission electron microscopy.

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https://dx.doi.org/10.1021/acsbiomaterials.0c01034
ACS Biomater. Sci. Eng. 2021, 7, 114−121