Materials Science and Engineering C 39 (2014) 299–304

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Electrochemical and cellular behavior of ultrafine-grained titanium in vitro

H. Maleki-Ghaleh [a], K. Hajizadeh [a], A. Hadjizadeh [b], M.S. Shakeri [c], S. Ghobadi Alamdari [a], S. Masoudfar [a], E. Aghaie [d], M. Javidi [e], J. Zdunek [f,*], K.J. Kurzydlowski [f]

[a] Faculty of Materials Engineering, Sahand University of Technology, Tabriz, Iran
[b] Department of Biomedical Engineering and Center of Excellence on Biomaterials, Amirkabir University of Technology, Tehran, Iran
[c] Materials and Energy Research Center, Karaj, Iran
[d] Department of Materials Science and Engineering, Saveh Branch, Islamic Azad University, Saveh, Iran
[e] Department of Materials Science and Engineering, School of Engineering, Shiraz University, Shiraz, Iran
[f] Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland

ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 21 January 2014
Accepted 1 March 2014
Available online 10 March 2014

Keywords:
Equal channel angular pressing (ECAP)
CP-Ti
Ultrafine-grained materials
Corrosion resistance
Cell behavior

ABSTRACT

The electrochemical and cellular behavior of commercially pure titanium (CP-Ti) with both ultrafine-grained (UFG) and coarse-grained (CG) microstructure was evaluated in this study. Equal channel angular pressing was used to produce the UFG structure titanium. Polarization and electrochemical impedance tests were carried out in a simulated body fluid (SBF) at 37 °C. Cellular behaviors of samples were assessed using fibroblast cells. Results of the investigations illustrate the improvement of both corrosion and biological behavior of UFG CP-Ti in comparison with the CG counterpart.

1. Introduction

Titanium and its alloys, e.g. Ti–6Al–4V have been widely used as implant materials due to their excellent mechanical and biological performance [1]. Although commercially pure titanium (CP-Ti) is biologically more compatible than Ti–6Al–4V, in its coarse-grained (CG) state CP-Ti does not have strength high enough for the most structural applications [2]. On the other hand strength of pure titanium can be increased by grain-size refinement induced by severe plastic deformation (SPD) [3]. Numerous reports over the last decade have firmly established equal channel angular pressing (ECAP) as the promising method for enhancing strength of pure titanium through refining its grain size below <1 μm [4,5]. The principle of ECAP is illustrated schematically in Fig. 1 in the form of a section through a die. The die is constructed with two channels, equal in cross-section, which intersect at an angle of Φ and there is also an additional angle, Ψ, which defines the arc of curvature at the outer point of intersection of the two channels. The test sample is machined to fit tightly within the channel and it is pressed through the die under a load P using a plunger. In practice, the same sample may be pressed through the die several times to increase the level of the imposed strain [6].

In addition to the mechanical strength corrosion resistance is another key property of materials that could be affected by the SPD processing. This property is of great importance particularly when the UFG material is to be used in biomedical applications. Thus the material processed by SPD has to be evaluated electrochemically to confirm its corrosion behavior. It should be noted in this context that because of small grain size, ultrafine-grained materials processed by SPD methods contain a large volume fraction of grain boundaries [3] which are an important factor altering corrosion resistance [7], which in UFG structures is usually accelerated [8]. There is also a tendency for localized corrosion which is a problem limiting the use of medical implants [9] which could be solved using the nanostructures with high passivation process [10]. On the other hand, passivating layers may change biological behaviors of implants [11–13]. Thus, in the present investigation, the electrochemical and biological properties of both UFG and CG titanium have been evaluated.

2. Material and experimental procedure

2.1. ECAP procedure

The chemical composition (wt.%) of the CP-Ti investigated was 0.18% O, 0.03% N, 0.16% Fe, 0.17% Pd, and 0.18% Cr and balanced with Ti. The supplied material was annealed at 800 °C for 1 h in argon

* Corresponding author at: Warsaw University of Technology, Materials Science and Engineering, Faculty Woloska, 141 02-507, Warsaw, Poland.

http://dx.doi.org/10.1016/j.msec.2014.03.001
0928-4931/

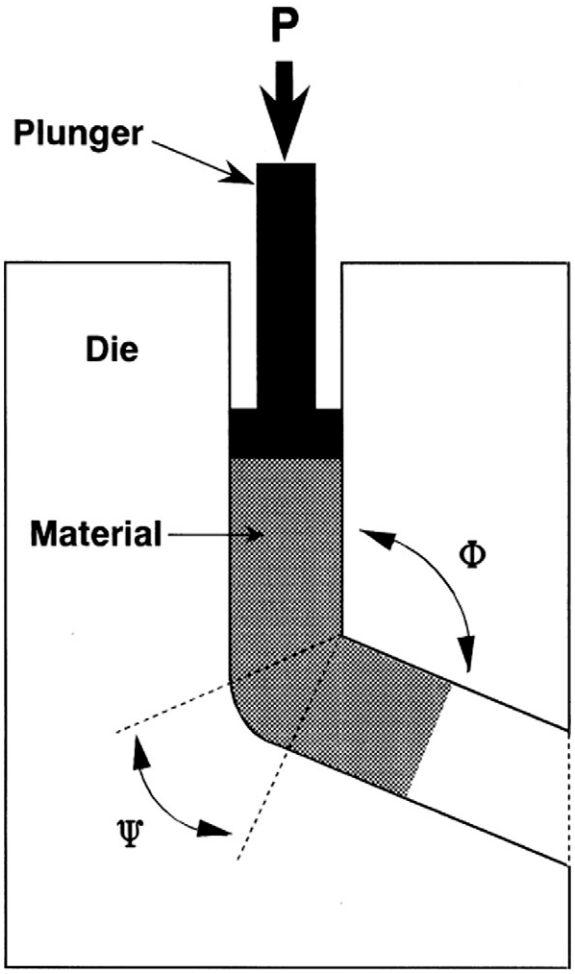

Fig. 1. The principle of equal channel angular pressing through a die, showing the two angles Φ and Ψ.

atmosphere and then air cooled. This resulted in an equiaxed microstructure with an average grain size of about 20 μm, as shown in Fig. 2.

ECAP samples with a 70 mm in length and diameter of 14.5 mm were machined from the annealed material. These samples were subjected to ECAP in a die with the channel angle $\Phi = 105°$ and the corner angle $\Psi = 20°$ which led to an imposed strain of about 0.8 for each pass. ECAP was performed up to 10 passes with route B_C at 250 °C.

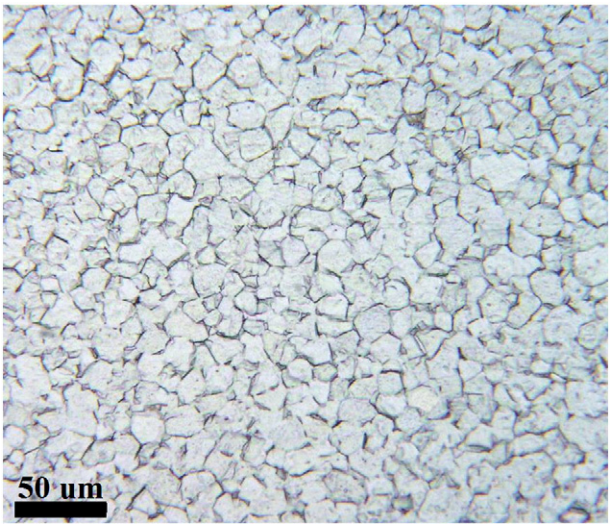

Fig. 2. Micrograph of CP-Ti in the as-annealed condition — LM.

Following the ECAP process, disk-shape samples (14.5 × 2 mm) were cut perpendicular to the direction of ECAP die exit channel. Metallographic samples were prepared by wet grinding up to 5000 grit using SiC papers followed by cleaning successively in acetone and ethanol using an ultrasonic bath for 30 min.

2.2. Electrochemical behavior

Electrochemical behavior of Ti samples was evaluated by polarization tests and electrochemical impedance spectroscopy (EIS) using a potentiostat/galvanostat ZAHNER model 96317 Kronach. The tests were performed at 37 °C in SBF. A saturated calomel and a platinum plate were used as the reference and auxiliary electrodes, respectively. The SBF solution was prepared according to Kokubo instruction as shown in Table 1 [14]. The samples were immersed in SBF solution for 1 h at open circuit potential (OCP) before the measurement. The polarization tests were performed in the range from −1100 to +2000 mV with scan rate of 1 mV/s. The EIS tests were carried out at open circuit potential by applying a sine wave of 5 mV amplitude. The applied frequency was chosen in the range of 100 KHz to 10 mHz. Impedance spectra were reported via Nyquist and Bode Diagram.

2.3. Cellular behavior

Biocompatibility of the samples was tested using the mouse fibroblasts L929 cells. The Ti samples, sterilized using Gamma radiation, were placed into the well plates (Falcon, USA). The cells were plated on the samples at a seeding density of 5.0×10^4 cells/ml using Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and antibiotics (100 U/cm^3 penicillin G, 0.1 mg/cm^3 streptomycin, Invitrogen). A 5 ml of the DMEM medium was added to each well and the culture dishes were incubated for 6 days at 37 °C under an atmosphere of 5% CO_2, with medium exchanges every 2 days. Fibroblast proliferation on the titanium samples was determined at 2, 4, and 6 days using an MTT assay (Sigma-Aldrich).

2.4. Characterization

The microstructure of ECAP-ed specimen was investigated by transmission electron microscopy (TEM) with a Philips CM 200 electron microscope operated at 200 kV. Specimens for TEM were cut from the middle sections of the ECAP-ed billets perpendicular to the pressing direction (cross section). Thin foils were first mechanically polished and finally electropolished in a Tenupol 5 double jet polishing unit in a solution of 5 vol.% $HClO_4$ in methanol at −30 °C.

Surface morphology of the samples was studied using optical microscope (OM) (Olympus, PMB3) and SEM (Cam Scan MV2200, Vega Tescan, Czech Republic). Surface wettability of the UFG and CG samples

Table 1
The composition of SBF fluid [14].

Salt	Amount	Purity %	Molecular weight (g/mol)
NaCl	8.036 (g)	99.5	58.44
$NaHCO_3$	0.352 (g)	99.7	84.01
KCl	0.225 (g)	99.0	74.56
$K_2HPO_4 \cdot 3H_2O$	0.230 (g)	99.0	228.23
$MgCl_2 \cdot 6H_2O$	0.311 (g)	99.0	203.30
1 M HCl	40 (ml)	–	–
$CaCl_2$	0.293 (g)	98	110.98
Na_2SO_4	0.072 (g)	99.0	142.04
Tris	6.063 (g)	99.8	121.14
1 M HCl	0.0–0.2 (ml)	–	–