Synthesis and Properties of Ag-doped Titanium-10 wt% 45S5 Bioglass Nanostructured Scaffolds

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Abstract A new kind of biomedical Ti-45S5 Bioglass-Ag nanocomposites and their scaffolds with antibacterial function was developed by the introduction of 1.5 wt% Ag into the Ti-10 wt% 45S5 Bioglass matrix. The microstructure, hardness and corrosion resistance in Ringer solution of the Ag-doped Ti-45S5 glass were investigated. The Vickers hardness of the bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposites reached 480 HV_{0.3}. Contact angles of water on the microcrystalline Ti and nanostructured Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag sample were determined and show visible decrease from 55.2° to 49.6°. In vitro tests culture of normal human osteoblast cells showed very good cells proliferation, colonization and multilayering. In vitro bacterial adhesion study indicated a significantly reduced number of bacteria (*Staphylococcus aureus*) on the bulk nanostructured Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag plate surface in comparison with that on microcrystalline Ti plate surface. Development of porous Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffolds aims in enhancing bone ingrowth and prosthesis fixation.

KEY WORDS: Biomaterials; Nanocrystalline materials; Titanium; 45S5 Bioglass

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1 Introduction

A noticeable increase in the application of Ti-based implants is evidenced in the recent years, although those solutions are not always problem free [1]. Modification of its chemical composition is one of the methods allowing the change of biological properties of Ti and its alloys [2]. Current goals in the development of new Ti-based biomaterials are (1) to avoid potentially toxic elements, such as vanadium, and (2) to produce titanium alloys with a high fatigue strength but a low Young's modulus comparable to cortical bone ($E = 10\text{--}25$ GPa) [3]. The β -titanium alloys partially fulfill these requirements and are currently used for implant materials.

The other way is to produce a composite that will exhibit the favorable mechanical properties of titanium and excellent biocompatibility and bioactivity of ceramic [4]. There are numerous bioceramics currently in use in both dentistry and medicine [5, 6]. Bioactive glasses and glass ceramics are available for use in dentistry under various

trade names. Additionally, porous ceramics such as calcium phosphate-based materials have been used for filling bone defects.

Composite materials/nanomaterials containing titanium (Ni-free stainless steel) and bioceramic as a reinforced phase are expected to have broad practical applications [4, 7–10]. The current projects aim to fabricate Ti-based porous scaffolds to promote bone or tissue ingrowth into pores and provide biological anchorage. Generally, porous metallic scaffolds are fabricated using a variety of processes to provide a high degree of interconnected porosity to allow bone ingrowth. Fabrication technologies include chemical vapor infiltration to deposit tantalum onto vitreous carbon foams, solid freeform fabrication, self-propagating high-temperature synthesis and powder metallurgy [7, 11–13]. While these porous metals have been successful at encouraging bone in growth both in vivo and clinical trials, the range of materials and microstructures available is still rather limited.

Various methods are used in an effort to improve the interfacial properties between the biological tissues and the existing implants, e.g., Ti and Ti-based alloy. The electrochemical technique can be used as a potential alternative for producing porous Ti-based metals for medical implants [14]. Good corrosion resistance of the titanium is provided by the passive titanium oxide film on the surface. This layer is important for the good biocompatibility. Porous implants layer has lower density than respective bulk, and good mechanical strength is provided by bulk substrate. Hence, the latter is attractive with respect to bulk titanium alloys. The porous layer on the Ti substrate is necessary for osseointegration with bone, which is not normally provided by the native oxide. Those new approaches are the gateway for traditional industry to nanotechnology and knowledge-based materials, with positive effects on health issues [15, 16]. Till now, a significant number of studies have focused on the effect of surface topography on cell functions such as adhesion, morphology, cytokine release and differentiation [16].

From the viewpoint of biomedical engineering, silver possesses many advantages, such as good antibacterial ability, excellent biocompatibility and satisfactory stability [17, 18]. Recently, the antibacterial activity of the $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ with $x = 0$ and 0.2 was studied [19]. The Ag:HAp nanopowders show strong antibacterial activity. The Ag^+ partially substitutes calcium and enters the structure of hydroxyapatite.

The purpose of this study was to develop a new Ti-10 wt% 45S5 Bioglass porous scaffold nanocomposites by silver addition in order to improve its antibacterial function for medical application. To investigate the properties of Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite scaffolds, compositional analysis, microstructural observations,

phase identifications, microhardness, corrosion resistance studies and cytotoxicity evaluation were performed. Yet to the author's knowledge, there are no papers regarding the addition of Ag into titanium-10 wt% 45S5 Bioglass nanocomposite to be appeared till now.

2 Materials and Methods

2.1 Sample Preparation

The Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite was prepared by mechanical alloying (MA) and powder metallurgy process. The mixed powders were first blended by MA process under argon atmosphere using a SPEX 8000 Mixer Mill for 20 h and then uniaxially pressed at compacting pressure of 600 MPa. Finally, the green compacts (8 mm in diameter and 3 mm in height) were heat-treated at 1,150 °C for 2 h under an argon atmosphere (99.999% purity) to form ordered phases.

Additionally, the blended Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag was mixed with ammonium hydrogen carbonate (NH_4HCO_3), which was used as the space-holder material. The size of space-holder particles was 500–800 μm . The mixture of an amorphous Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag powder and NH_4HCO_3 was uniaxially pressed at compacting pressure of 400 MPa. The green compacts (8 mm in diameter and 3 mm in height) were sintered in a vacuum of 1.33×10^{-2} Pa in two steps: (1) 175 °C for 2 h to burn out the space-holder particles; (2) 1,150 °C for 10 h. Porous Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag composites with porosities of 48, 67 and 72% were fabricated by adding 30, 50 and 60 wt% ammonium hydrogen carbonate to the powder mixture (see Fig. 1f).

For all tests, the disks of the synthesized samples as well as microcrystalline Ti (grade-2) were polished with 1,000 SiC paper in water and ultrasonically rinsed with acetone.

2.2 Materials Characterization

The phase constitution of Ti-45S5 Bioglass-Ag scaffolds was analyzed by X-ray diffraction with CuK_α radiation. The grain sizes were estimated by Scherrer method. Scanning electron microscope (SEM) with energy dispersive spectrometer (EDS) was used to characterize the pore structure and the chemical composition of the prepared scaffolds. The density of the sintered samples was determined using Archimedes method. The Vickers' microhardness of the bulk samples was measured using microhardness tester on the polished surface under a load of 300 g.

The surface morphology of the samples was investigated using a VegaTescan SEM equipped with an EDS and an

Fig. 1 XRD spectra of Ti, 45S5 Bioglass (10 wt%) and silver (1.5 wt%) powders mechanically alloyed for different times: *a* Ti—0 h, *b* 45S5 Bioglass—0 h, *c* Ag—0 h, *d* 15 h of MA, *e* bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite after annealing at 1,150 °C for 2 h, *f* ammonium hydrogen carbonate (NH_4HCO_3)—the space-holder material, *g* Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite scaffold with porosity of 67% after sintering in a vacuum of 1.33×10^{-2} Pa in two steps: at 175 °C for 2 h and at 1,150 °C for 10 h

Optical Profiler Veeco WykoR[®] NT 1100. Using the 3D optical profiler, the following standard roughness parameters were estimated: R_a is arithmetic mean roughness (μm), R_t is maximum height of the profile (μm), R_z is ten point mean roughness (μm), S_{sc} is hybrid 3D parameters: mean summit curvature (μm^{-1}), S_{dq} is root mean square (degrees), and S_{dr} is surface area ratio gradient (%).

For corrosion measurements, the Solartron 1285 potentiostat was applied. The corrosion resistance of different samples was measured in Ringer's solution [20] applying potentiodynamic mode with scan rate 0.5 mV/s at temperature of (37 ± 1) °C, controlled by thermostat. The corrosion test was run in EG&G K0047 corrosion cell. The corrosion potentials (E_C) and corrosion current densities (i_C) were estimated from the Tafel extrapolations of the corrosion curves, using CorrView software.

For the surface wettability measurements, contact angles θ for each group pair were determined by computer software analyze Musial (Poland) from geometrical shape of droplets recorded by the optical system with digital camera. Droplets' placement was realized by a special micro-pipette with a constant volume of test liquid (2 μL) on clear, polished and rinsed with alcohol surface, but it raises until the bottom touches the specimen. If the drop is large enough, the adhesion to the surface pull it off from the tip. All the data were obtained 10 s after placing the droplet on the surface under ambient condition, procedure was repeated three times. Possible measuring errors for θ may be caused by different shapes of liquid drops placed on the investigated samples surfaces, possible interactions, as well as from different liquid vaporization rates and inaccuracy in graphical interpretation of the pictures with the use of the computer software. For each recorded picture (i.e., for each liquid drop), the geometrical shape analysis was repeated 10 times: The extreme values were rejected, and the arithmetic mean value was calculated for the accepted findings.

2.3 In Vitro Tests

2.3.1 Cytotoxicity Test in Static Conditions

For this test, the synthesized Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposites, their scaffolds with 67% porosity and microcrystalline Ti disks were used. These disks were sterilized by autoclaving at 120 °C for 15 min and were separately located at the bottom of 24-well microplates. Normal human osteoblast (NHOst) cells from Cambrex (CC-2538) were cultured onto each disk at a concentration of 5,000 cells per well in 1 mL of culture medium under static conditions. The cells were cultured at 37 °C in a 5% CO_2 incubator for 1 and 5 days. Then, the cells were fixed with a 25% glutaraldehyde solution for 10 min and stained with a 10% Giemsa's staining solution for 10 min. The specimens were sputter-coated with gold and examined using scanning electron microscopy.

2.3.2 In Vitro Antibacterial Activity

In this study, the strain of *Staphylococcus aureus* (ATCC 6538) was assessed. Cultures of *S. aureus* were obtained from commercial sources (American Type Culture Collection), and an aliquot was inoculated into 5 mL of tryptic soy broth (TSB) and incubated overnight. The resulting log-culture had a concentration of $\sim 10^9$ CFU/mL. The experimental samples (typical dimensions were $d = 8$ mm in diameter and $h = 3$ mm in height) were placed in 5-mL sterilized tubes followed by the addition of 0.5 mL of the bacterial suspension to TSB. The tubes were incubated at

37 °C with shaking at 200 r/min for 3 h. At the end of the incubation period, the samples were gently rinsed three times with PBS in order to eliminate the non-adherent bacteria. Then, the samples were put into individual new tubes containing 5 mL of PBS each and then subjected to sonication at frequency of 20 kHz (Bandelin Sonoplus) for 5 min, followed by additional vortexing for 60 s to remove the adhering microorganisms. The viable organisms in the buffer were quantified by plating serial dilutions on tryptic soy agar (TSA) plates. TSA plates were incubated at 37 °C, and the colony-forming units were counted visually.

2.3.3 Statistics

All experiments were repeated three times. Commercially available program Excel (Microsoft, WA) and statistical software R version 3.0.1 were used to determine whether any significant difference existed in bacterial number in the antibacterial experiments. Analysis of variance followed by Tukey's honest significant difference test was performed on the bacterial counts. The statistical significance was defined as $p < 0.05$.

3 Results

In the present work, bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite and Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffolds with different porosities (48, 67 and 72%) were produced.

3.1 Structure, Hardness and Corrosion Properties

X-ray diffraction was employed to study the effect of MA on Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag composites. In the MA process, the mixtures of pure elemental powders were milled for 15 h to achieve the Ti-45S5 Bioglass-Ag nanocomposites. Figure 1a–c shows the XRD patterns of the starting microcrystalline titanium (ICDD: 5-682), amorphous 45S5 Bioglass and silver (ICDD: 4-783)

powders. After 15 h of milling, the starting materials have almost entirely transformed into an amorphous phase (Fig. 1d).

XRD analysis of Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold with porosity of 67% showed the presence of α -Ti-type structure with cell parameters $a = 0.2970$ nm, $c = 0.4759$ nm (Fig. 1g). When 10 wt% of 45S5 Bioglass is added, the volume of the hexagonal structure of Ti increases, as manifested by a shift of the diffraction peaks of the (1 0 0), (0 0 2), (1 0 1) and (102) crystal planes of titanium toward smaller angles in comparison with pure microcrystalline titanium. Calcium and sodium have higher atomic diameters in comparison with titanium. According to the Scherrer method of XRD profiles, the mean crystallite size of heat-treated Ti-45S5 Bioglass-1.5 wt% Ag nanocomposites was 9 nm.

The results of EDS analysis of the surface of sintered Ti-10 wt% 45S5 Bioglass-1.5 wt% nanocomposite are shown in Fig. 2. It can be confirmed that synthesized scaffold mainly consists of titanium matrix with elements of Ag, O, Si, Ca, Na and P. The presence of the iron atoms in Ti-10 wt% 45S5 Bioglass-1.5 wt% nanocomposite could be explained by Fe impurities trapped in the MA powders from erosion of the milling media. Almost homogenous distributions of Ag particles are detected with an average size of approximately 0.3 μ m (Fig. 2).

Scanning electron micrographs of the Ti-45S5 Bioglass-Ag nanocomposite scaffolds with different porosity are shown in Fig. 3. The Ti-10 wt% 45S5-1.5 wt% Ag scaffolds with porosities of 48% (Fig. 3a), 67% (Fig. 3b) and 72% (Fig. 3c) have complex microstructured surfaces. For example, Ti-10 wt% 45S5-1.5 wt% Ag scaffold with porosity of 72% exhibited wide cavities, 80–300 μ m in diameter. The smooth Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag surface is presented in Fig. 3d. The relative density of the bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite was observed to be 94.0%.

Earlier, it has been shown that the sintered Ti-hydroxyapatite nanocomposites exhibit various distribution of the Vickers' microhardness, corresponding constitutional

Fig. 2 EDS surface spectrum of the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite with distribution of Ag particles

Fig. 3 Scanning electron micrographs of the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite scaffolds with porosities of 48% (a), 67% (b), 72% (c); the surface roughness of the bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite is shown in (d)

Table 1 Structural parameters, microhardness, mean values of corrosion current densities and corrosion potentials of studied bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite (B) and Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite scaffold with 67% porosity (C) in comparison with microcrystalline titanium (A)

Sample	a (10^{-1} nm)	c (10^{-1} nm)	V (10^{-3} nm ³)	HV _{0.3}	i_c (A cm ⁻²)	E_c versus SCE (V)
A	2.948	4.784	36.00	180	2.27×10^{-6}	-0.36
B	2.968	4.761	36.32	480	3.50×10^{-7}	-0.43
C	2.970	4.759	36.35	–	7.15×10^{-6}	-0.44

change and hydroxyapatite content [4]. The Vickers' hardness for Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposites reaches 480 HV_{0.3} and is higher than of pure microcrystalline Ti metal (180 HV_{0.3}). Table 1 shows the effect directly connected with structure refinement and obtaining of nanostructure.

The addition of 45S5 Bioglass to titanium has also a positive effect on the corrosion resistance of titanium in Ringer's solution [7]. Corrosion resistance potentiodynamic test in Ringer solution at 37 °C shows no negative effect of porosity on Ti-10 wt% 45S5 Bioglass-1.5 wt%

Ag scaffolds in comparison with microcrystalline Ti (Table 1; Fig. 4). Titanium composites with 10 wt% of 45S5 Bioglass and 1.5 wt% Ag have better corrosion resistance ($i_c = 3.50 \times 10^{-7}$ A/cm², $E_c = -0.43$ V) than microcrystalline titanium ($i_c = 2.27 \times 10^{-6}$ A/cm²).

3.2 Surface Properties

Figure 5a, c shows optical profiler 3D topography of polished bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite and Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag

Fig. 4 Potentiodynamic polarization curves of: microcrystalline titanium *a*, Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite scaffold with 67% porosity *b*, bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite *c* in Ringer's solution at 37 °C

scaffold with 67% porosity. Different topographies of the samples are shown in Fig. 5a, c. The R_a , R_t and R_z values for the bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite were approximately 0.92, 9.05 and 7.45 μm , respectively (Table 2), whereas the roughness parameters for the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold increased (Fig. 5c). The scaffold with 67% porosity had an

average surface roughness with R_a , R_t and R_z values in the range of 73–422 μm . Figure 5b, d shows the X-line profiles of the studied materials. Large pore formation, supporting cell growth and proliferation, occurs during space-holder sintering process.

The hybrid 3D parameters (S_{sc} , S_{dq} , S_{dr}) for scaffold materials show similar tendency as the roughness parameters. S_{sc} is the average of the principal curvature of the local maximums of the surface, and therefore can be helpful in predicting friction and wear. S_{sc} values differ from the polished bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag sample and its scaffold. The S_{dq} value that affects the wetting of the surface by fluids significantly increases for the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold sample. An increase in the surface slope (S_{dq}) may enhance the fixing force of the cells, resulting in a more stable connection between the implant and bone [21]. Finally, S_{dr} , which expresses the increase in the interfacial surface area relative to the area of the projected (flat) x , y plane, may improve the area for the attachment of cells. In our study, there is a considerable increase in S_{dr} for the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold surface compared to the polished surface.

As shown in Fig. 6, surface wettability assay recorded a lower water contact angle of Ti-10 wt% 45S5 Bioglass-

Fig. 5 Optical profiler 3D topography (229.1 × 301.1 μm scan size) (a, c) and X-profiles (b, d) of polished bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite (a, b), the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold with a 67% porosity (c, d)

Table 2 Roughness and topography surface parameters for the studied samples on different processing routes; parameters taken from surface area of 0.069 mm²

Sample	R_a (μm)	R_t (μm)	R_z (μm)	S_{sc} (μm ⁻¹)	S_{dq} (degrees)	S_{dr} (%)
A ^{a,b}	0.21	6.35	4.43	2.71	37.12	23.86
B ^b	0.92	9.05	7.45	0.84	27.95	12.91
C	73.01	433.03	421.57	11.07	80.42	11,223.21

^a Data from ref. [25]; ^b polished **Fig. 6** Contact angles of distilled water on the microcrystalline Ti (a), bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite (b)

1.5 wt% Ag bulk nanocomposite ($49.6^\circ \pm 0.4^\circ$) than that of the microcrystalline titanium ($55.2^\circ \pm 0.8^\circ$), suggesting that nanostructured composite exhibited the enhanced surface hydrophilicity.

3.3 In Vitro Cytocompatibility

Significant differences in the osteoblast culture can be observed, on the samples of bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite, after the first and fifth day in the in vitro cytocompatibility test (Fig. 7). After the first day of incubation, good adhesion of the cells to the surface of the tested sample is noticed, with the formation of long filopodia (Fig. 7a). Moreover after the fifth day of incubation, the sample was covered with a compact cell layer (Fig. 7b).

However, a significant difference has been observed after 1 day, in the cell growth, culturing the cells on the Ti-

10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold with 67% porosity. The cells entered the pores on the surface rather than forming filopodia (Fig. 7c). After fifth day of cell culture, the cells on the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold were noticed to be well spread on the surface of the porous scaffold as well as to be penetrating the pores (Fig. 7d). Topography and roughness of the surface affect cell attachment and proliferation [2]. Adhesion of the cells on the surface of the implant is improved by the surface irregularities such as pores, protrusions and hillocks, acting as anchorage for the cells.

3.4 In Vitro Antibacterial Test

Figure 8a shows the results of viable bacteria adhering to the different experimental material surfaces when exposed to *S. aureus*. Figure 8b illustrates the representative macroscopic images of viable adherent bacteria. Bacterial adhesion was significantly reduced on Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite when compared to the samples without silver. The Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposites were observed to have significantly lower adhesion of *S. aureus*, suggesting that the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposites were antibacterial.

4 Discussion

The main purpose of current research is to prevent the failures caused by infection by changing the biomaterial's properties and making them highly friendly for surrounding tissues. In this work, the MA and space-holder sintering processes were used to synthesize nanostructured Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffolds. The high deformation during the MA leads to the large dislocation density and formation of the nanometer size subgrains. Nanostructured biomaterials with unique mechanical and surface properties are considered to be the future generation biomaterials [22, 23]. Nanograined materials with a very high number of atoms on the grain boundary have large grain boundary energy. Thus, they exhibit entirely different behavior, compared to the micron-sized grains.

Fig. 7 Scanning electron micrographs of osteoblasts cultured on bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite (a, b) and Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffolds with 67% porosity (c, d) after the first (a, c), the fifth day (b, d)

It has been proven that the surface properties of the titanium implants determine successful osseointegration. The results published recently by Sul *et al.* [24] showed that the morphology of different commercially available clinical titanium implants differed due to the surface modification techniques used during manufacture. The microcrystalline titanium presented a smooth surface with very low values of R_a , R_t and R_z [25]. The bulk Ti-10 wt% 45S5-1.5 wt% Ag Bioglass nanocomposite had R_a , R_t and R_z values of around 0.92, 9.05 and 7.45 μm , respectively. The Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold with 67% porosity had average surface roughnesses, with R_a , R_t and R_z in the 73–422 μm range. However, it has been documented that the pore size of approximately 200–500 μm is optimal for the cell adhesion, differentiation and ingrowth of osteoblasts [26]. There are other recent reports to prove that the small micropores support living cells growth [27].

Implants, due to the corrosive environment of the tissue and body fluids, may undergo unexpected local corrosion attacks, leading to a release of the corrosion products to the tissue and its poisoning (toxication). The corrosion test results indicated that the microcrystalline titanium possesses a lower corrosion resistance and consequently a higher corrosion current density ($i_c = 2.27 \times 10^{-6} \text{ A/cm}^2$,

$E_c = -0.36 \text{ V}$) in the Ringer's solutions. The bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag has a better corrosion resistance ($i_c = 3.50 \times 10^{-7} \text{ A/cm}^2$, $E_c = -0.43 \text{ V}$).

The surface wettability was confirmed to be a key parameter influencing the proliferation of osteoblasts [28, 29]. Enhanced surface wettability would improve the cellular adhesion by optimizing the protein adsorption and providing a suitable surface presented to the filopodia. As a result, the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag bulk, due to its good corrosion resistance and the surface wettability, should show a high extent of cell proliferation.

In order to test the toxicity of materials, as well as interaction between the tested materials, the *in vitro* cytocompatibility tests in static condition are conducted [30]. The first results of increased osteoblast adhesion on the nanostructured metals were presented by Webster and co-workers [15, 22, 27]. The grain boundaries favored the adhesion of the cells. A small degree of residual porosity after powder compaction also may have played a role in the cell adhesion. It was proposed that an increased area of the surface defects exposed to the cell culture and to a larger degree of surface electron delocalization caused enhanced cell adhesion. Independently, the different researches proved a remarkable increase in the pre-osteoblastic cells proliferation deriving from mice on commercial purity

Fig. 8 **a** Statistical results of viable adherent bacteria on microcrystalline titanium **A**, bulk Ti-10 wt% 45S5 Bioglass nanocomposite **B**, and bulk Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite **C**, **(b)** representative macroscopic photos of viable adherent bacteria on difference experimental material surfaces

(grade-2) titanium processed by ECAP [31]. Reducing the average grain size from 4.5 to 200 nm causes an increase in cell proliferation by a factor of almost 20 as well as the increase in the relative number of viable cells. Moreover, other study showed that the surface occupancy of mice fibroblast cells on nanostructured titanium (grade 4) increased from 53 to 872% after 3 days of culturing [32].

In this work, in vitro cytocompatibility test under static conditions was performed on the samples with different surfaces. The surface structure of the samples influences the intensity of cell growth on it. After 1 day, the osteoblasts that grew on the inserts adhered to the surface and after 5 days, covered most of it. Even after 1 day of cell culturing, a significant difference in the morphological characteristics of the cells on the porous and polished materials was visible. The cells tend to adhere with their entire surface to the porous sample penetrating the pores, whereas more spherical cells were seen with a smaller contact surface but more filopodia on the polished surface. After the first day, the cells strongly attached to the surface of the nanocomposite Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold with 67% porosity, not producing filopodia or only producing them in a smaller amount compared to the cell culture on the smooth surface. Therefore, it appears that the porosity of the material favors cell adhesion

especially of osteoblasts. After 5 days of cell culture, a smaller portion of the observed area was covered by cells on the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold in comparison with analogous bulk sample.

The inhibition of bacteria was investigated with the use of *S. aureus*. In vitro bacterial adhesion study indicated a significantly reduced number of *S. aureus* on Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag. When Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag material is immersed in body fluid, the metallic Ag particles at the surface of the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag could react with the body fluid and release ionized Ag into the surrounding fluid. A steady and prolonged release of the silver biocide in a concentration level (0.1 ppb) is capable of rendering antibacterial efficacy [33]. When there are enough Ag ions in the fluid, the Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite can exhibit antibacterial activity against bacteria.

Independent study showed that nanocrystalline Ti-10 wt% 45S5 Bioglass materials (scaffold and bulk) regardless to the form (powder or disk) do not affect the viability of *Candida albicans* and do not inhibit the growth of yeast in the direct or indirect contact [34]. Despite the fact that tested nanomaterials do not have negative influence against yeast, their structure and/or surface limits the growth of tested *Candida* yeast.

Fibroblasts have a crucial role in the initial step of the integration of implants with biological tissue. Recently, in vitro cytocompatibility of Ti-10 wt% 45S5 Bioglass nanocomposites and their scaffolds was evaluated [34]. These studies showed that regardless of the type of material tested (nano- or microcrystalline) samples did not show cytotoxic properties against cultured cells. Despite this, the intensity of the cell growth on the surface is dependent on the surface structure of the sample. Obtained results suggest that nanocrystalline Ti-10 wt% 45S5 Bioglass-1.5 wt% sample displays good cytocompatibility compared to microcrystalline titanium. Survival rate of CCD-39Lu fibroblasts was higher in the presence of the nanocrystalline material as compared to microcrystalline material in the same concentrations of the same intervals.

5 Conclusions

- (1) Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold with 67% porosity had average surface roughness in the 73–422 μm range.
- (2) The noticeable difference in the morphology of the NHOst cells on porous and polished materials is observed in SEM, even after 1 day of cell culturing.
- (3) Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite exhibits reduced bacteria adhesion (*S. aureus*) when compared to microcrystalline titanium.

- (4) The porous Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag nanocomposite scaffolds can promote bone or tissue ingrowth into pores and provide biological anchorage.
- (5) Chemical composition and the surface topography are key factors for the success of implant adaptation. Therefore the nanocrystalline Ti-10 wt% 45S5 Bioglass-1.5 wt% Ag scaffold may be an important step forward in the development of such a structure, which will support the process of osseointegration of the implant.

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