In vitro comparative investigation of bioactivity and biocompatibility behavior of titanium nano-composites fabricated by friction stir processing

Mohammad Javad Bathaei 1, Abbas Zarei-Hanzaki 1, Jhamak Nourmohammadi 2, Farahnaz Haftlang 1 and Hamid Reza Abedi 1

1 Hot Deformation & Thermomechanical Processing Laboratory of High Performance Engineering Materials, School of Metallurgy and Materials Engineering, College of Engineering, University of Tehran, Tehran, Iran
2 Faculty of New Sciences and Technologies, Department of Life Science Engineering, University of Tehran, Tehran, Iran
E-mail: zareih@ut.ac.ir

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Abstract
The aim of the present work is to fabricate the surface nano-composite through the friction stir process (FSP) followed by emphasizing on the comparative study from the surface characteristics (wettability and roughness), bioactivity and biocompatibility aspects. Toward this end, three different categories of conventional biocompatible powders include Hydroxyapatite, bioactive glass 45S5 and natural bone powder have been selected as second phase particles. Bone apatite formation, Mesenchymal stem cells (MSCs) adhesion and morphology have been characterized by scanning electron microscopy coupled with electron dispersive spectroscopy. The results of the contact angle demonstrate enhancing the hydrophilicity of titanium surfaces after surface modification due to the surface chemistry alteration. According to the immersion results in simulated body fluid, the surface modification by FSP enhances the bioactivity of titanium. Additionally, the biocompatibility of all the specimens except for titanium/hydroxyapatite (Ti/HA) composite is approved by MSCs culture. The unpleasant behavior of Ti/HA could be due to the changing the pH so it leads to cell death. Eventually, the MSCs are well-attached on the surface of titanium composites and possess polygonal morphology, while as a result of deviation from standard pH, cells get rounded on Ti/HA surface and cell death occurred.

1. Introduction
Commercially pure Titanium (CPT) has been utilized wide-ranging in bioapplications such as the dental implant, bone screws and plates due to the combination of appropriate property. Distinctive CPT properties including relatively low young modulus, and high corrosion resistance are frequently reported [1–3]. The application of untreated CPT is limited in loadbearing and orthopedic implants due to the poor cellular response, inappropriate osseointegration and low mechanical strength [4, 5].

Various kind of the biocompatible powders including alumina, calcium phosphates (e.g. Hydroxyapatite) and bioactive glass 45S5 have been utilized to modify the surface of CPT [6] through different surface engineering methods such as laser cladding [7], electrophoretic deposition [8], sol-gel [9] and plasma spraying [10]. Such modifications effectively improve the cell activity, however, the mechanical properties of the bulk material remains relatively unchanged. In recent years, there has been increasing interest in employing a thermo-mechanical process such as friction stir processing (FSP) in order to modify the surface of the materials in the composite state [11, 12]. During the FSP, a novel solid-state technique, the microstructure evolution occurs by the continuous frictional straining and rising the temperature during the movement of the rotational pin. In the microstructure of FSP treated materials, three distinct regions have been detected: (i) Stir zone (SZ) region which was severely affected by high temperature and severe strain by rotational pin and the grain
refinement is severe than other regions. Based on the ‘Hall-Petch’ equation, the grain size and yield strength are reversibly related to each other. The more decrease in grain size in the stir zone, the more increase in strength will occur in this region. (ii) the transitional region between the stir zone and the base metal, which is called thermo-mechanically affected zone (TMAZ), (iii) the base metal which not affected by strain and temperature, in this region the grain size is larger than SZ and TMAZ. According to the previous reports, the grain refinement micro-mechanism in regions ‘i’ and ‘ii’ is discontinuous dynamic recrystallization (DDRX) [13, 14]. Based on this micro-mechanism, grain boundary bulging initially occurs for nucleation of new dislocation free recrystallized grains [14]. Driving force for local boundary migration is provided by sub-grain formation. The necklace structure and subsequently the new fresh grains may form around the deformed grains as a result of the dislocation density inside the deformed grain [14]. These new grains grow inside the deformed grain due to the dislocation density difference hereupon grain refinement occurs. On the other hand, based on the ‘Zener mechanism’ and particle pinning effect of the second phase particle, further grain refinement can be achieved. Since the volume fraction of powder particles is the highest in the SZ (compared to the other regions), the Pinning effect of particles can hinder the grain boundary migration and slow down the grain growth particularly. This also may result in further grain refinement. Correspondingly, besides improving the strength and hardness of the materials, FSP can enhance the biocompatibility of the surface by using biocompatible micro-nano sized powders in order to fabricate surface composited biomaterial. Based on the literature, FSP provided the suitable substrate for osteoblast cell adhesion, proliferation, and expression of the integrin α1, the receptor proteins placed on the cell membrane resulting in improvement of the cell adhesion [15–17]. According to Zhu et al [17], the SiC particles were homogenously distributed in the FSP treated specimens resulting in the superior mechanical properties which enhanced the potential of the composite for load-bearing applications. According to the literature, the grain further grain refinement owing to the increase in the rotational speed of the pin, leads to the mechanical property improvement. This ultra-fine substrate along with the integration of the Mg elements in the matrix effectively boost the cellular adhesion of the surface [15]. Coming to the point, despite the various surface modification methods which have been employed to modify the surface of titanium; however, comparative investigation of the different categories of coatings and composites fabricated through FSP have been mainly overlooked.

In present work the surface of CP titanium has been modified though addition of biocompatible powders (nano-sized bioactive glass 45S5 (BAG), hydroxyapatite (HA) and submicron bone powder (BP)) in the course of FSP. The processed composites have been compared in respect of bioactivity and biocompatibility aspects. In this regard, immersion tests in SBF following by Scanning Electron Microscopy (SEM) and Electron Dispersive Spectroscopy (EDS) analysis have been conducted. Cell viability, Cell adhesion, and morphology characteristics have been investigated by in vitro tests (MTT assay) and SEM, respectively. The wettabiliy and roughness of the processed surface which significantly affect the cell behavior have been measured through contact angle test and profilometry.

2. Materials and methods

2.1. Initial materials

Pure titanium plates (grade 1; Total remainder elements <0.4, TIMET Co, Grenzano, Italy) with the dimension of 103 cm2 were used in this research with the aim of fabricating titanium surface composite, second phase particles such as bioactive glass 45S5 nano-sized powder (BAG) (<100 nm; Nic Ceram Razi Co, Isfahan, Iran) (45% SiO2, 24.5% Na2O, 24.5% CaO, 6% P2O5), hydroxyapatite (HA) (Ca10(PO4)6(OH)2) and bone powder (BP) were used in this experiment. Thermal decomposition method is utilized to prepare bone powder: initially, the bovine bone was washed and boiled in water for 1 h to be cleaned then heated to 900 °C for 2 h followed by 3 h room temperature ball milling with the speed of 350 rpm by alumina balls in order to gain bone powder. According to the literature [18], thermal decomposition is one of the inorganic methods to produce calcium phosphate from the bovine bone.

2.2. Surface modification of titanium by FSP

The surface of titanium plates was grounded by 120 sandpaper subsequently in order to insert the powders on the surface of titanium, holes were machined on the titanium surfaces with depth, diameter and constant interval of 2.5 mm, 2 mm and 3 mm, respectively. Then holes were cleaned in an ultrasonic bath contains ethanol and then poured with each powder with equal mass. The rotation speed of 800 rpm, the travel speed of 315 mm min−1 and tilt angle 0° were selected as FSP parameters and each titanium plates were one pass processed as is shown in figure 1. This process was conducted by a conic shape tungsten carbide pin with the diameter and height of 20 mm and 3 mm, respectively.
2.3. Microstructural characterizations
The powders used for surface composite formation were examined by x-ray diffraction with CuKα radiation at 40 kV and 30 mA. In order to investigate the microstructural study, three categories of powders were Gold-sputter coated before the characterization by scanning electron microscopy (SEM) analysis. The cross-sectional cut has been performed followed by surface preparation including grinding by 120–5000 SiC papers and polishing with alumina-ethanol suspension. The mixture of 85 ml H2O + 10 ml HF + 15 ml HNO3 has been used as the etchant solution for 90 seconds. Field Emission Scanning Electron Microscopy (FESEM) (FEI NOVA NANOSEM 450) coupled with Energy Dispersive Spectroscopy (EDAX) (BRUKER X Flash6l 10) were utilized for surface study and cross-sectional observations.

2.4. Contact angle test
After surface preparation, the specimens were cleaned in an ultrasonic bath with acetone solution for 20 min and dried in air. In order to assess the wettability of specimen surfaces, the contact angle for specimens was measured with water droplets at room temperature. The contact angle measurement system was conducted with WCA; OCA 20, Data physics Instruments and that was equipped with droplet dispenser, digital camera, and image analysis software. The distilled water drop was placed on the surface by syringe, in the next, drop contact angle was measured by software after waiting for the 20s. All the experimental procedures mentioned in this section were repeated for the third time to confirm the results.

2.5. Profilometry
The surface roughness parameter (Ra) was measured by a profilometer (T-8000 Hommelwerke) conducted in a 3 mm width from the process region with the standard cut-off 0.8 mm and speed of 0.05 mm s$^{-1}$. Each test was reported at least 3 times and the average was reported.

In order to provide specimens for in vitro tests and cell studies, the specimens with the dimension of 3*5*5 mm$^3$ were cut out from the uppermost region of the surface of different treated specimens.

2.6. In vitro apatite formation and characterization
With the aim of comparative study, titanium composite surfaces bioactivity, each sample was immersed in 10 ml of simulated body fluid (SBF) at 37°C in the incubator (Memmert, Germany) for 14 days then was washed by distilled water and dried before SEM characterization. SBF solution with the composition of NaCl (3.996 g), NaHCO3 (0.175 g), KCl (0.112 g), K2HPO4·3H2O (0.114g), MgCl2·6H2O (0.1525 g), Na2SO4 (0.0355 g), and CaCl2 (0.139 g) were dissolved in 500 ml deionized water during stirring with magnetic stirrer and then solution pH should be reduced to 7.4 by adding Hydrochloric acid (HCl) [19]. After immersion, processed surface was characterized with FESEM (Hitachi S4160) coupled with EDAX (BRUKER X Flash6l 10).

2.7. Primary cell culture
Before applying cell culture assay, specimens were sterilized ultrasonically with 100% acetone solution for 30 min and both specimens and cell culture equipment were sterilized with an autoclave. Mesenchymal Stem cells (MSCs) from National Cell Bank of Iran (NCBI; Pasteur Institute) were cultured in the Dulbecco’s Modification of Eagles Medium (DMEM; BIO-IDEA; Iran) containing 10% fetal bovine serum (FBS; BIO-IDEA; Iran), 100 mg ml$^{-1}$ streptomycin (Sigma; USA) and 100 mg ml$^{-1}$ penicillin (Sigma; USA). The incubation was carried out at 37°C and the atmosphere contains 5% CO2 with 100% humidity.
2.8. MTT assay (Cell viability and proliferation)

MTT assay is a cell metabolic activity based test that was used to evaluate cell viability and proliferation. BIO-IDEA MTT assay kit was used to assess the viability and proliferation of MSCs. The cells were seeded on specimen surfaces at a density of $1 \times 10^4$ cm$^{-2}$ then the culture plates were incubated for 4 days. After incubation time, the culture medium was rinsed with Phosphate Buffered Saline (PBS; BIO-IDEA; Iran) solution to remove remained cells and replaced with 10 μL of MTT stock solution (12 mM). After that, they kept
in the incubator for 4 h. In order to dissolve blue insoluble crystals of formazan which was produced by the living cell as the result of cell metabolic activity, 50 μl of dimethyl sulfoxide (DMSO; BIO-IDEA; Iran) was added. After 10 min the solution was transferred to the new 48-well plate microplate reader. Finally, optical density was measured by an enzyme labeling instrument using a spectrophotometer at a wavelength of 570 nm (Stat Fax-2100, USA).

2.9. Cell adhesion and morphology assay
The MSCs with the $5 \times 10^4$ per cm$^2$ were cultured on pure titanium and treated specimens which were sterilized ultrasonically and washed with Phosphate Buffered Saline (PBS; BIO-DEA; Iran). The cells were fixed by 4% glutaraldehyde after 4 days of culturing on the surfaces. The cells were dehydrated with an alcohol solution (10% ethanol increments; each step 10 min) after extensive washing in PBS and deionized water. Finally, each titanium surfaces were sputter-coated by Gold target and cell morphology was analyzed by FESEM (Hitachi S4160).

2.10. Statistical analysis
Reasonable quantitative data of specimens were achieved by performing more than three independent experiments and the final result was shown as mean value ± standard deviation. A one-way analysis of variance (ANOVA) was used to compare any significant differences by StatPlus software and the statistical significance level of $p < 0.05$ was considered.

Figure 4. The cross-sectional SE (a) and BSE (b) SEM micrographs of the FSPed Ti/HA and the corresponding EDS elemental maps from the stir zone (c-f).
3. Results and discussion

3.1. Specimen characterization

In order to investigate the crystal structure of the experimented powders, x-ray Diffraction (XRD) has been utilized and the corresponding results are presented in figure 2. The XRD pattern of HA in figure 2(a) indicates its semi-crystallinity due to the existence of some sharp peaks and its structure has not amorphous completely. HA pattern confirms the presence of hexagonal-dipyramidal crystalline phase of HA and it has been reported by increasing the temperature, nucleation, and growth of hexagonal-dipyramidal nanocrystals increases and is stable up to 1200°C [20]. BAG spectrum shows a very broad peak characteristic of amorphous material (figure 2(b)). According to Qizhi Z. Chen et al [21, 22], XRD analysis of sintered BAGs at 1000°C for 1 h shows the hydroxyapatite and Na$_2$Ca$_2$Si$_3$O$_9$ crystalline phases and in fact, crystallization occurs at this temperature in BAG structure. They reported full crystallinity in BAG 45S5 could not be detected because full crystallization of the Na$_2$Ca$_2$Si$_3$O$_9$ phase requires too much CaO, however, CaO amount depleted when crystallinity reached to 80.7% [21]. The pattern for BP (figure 2(c)) shows the considerable number of peaks confirming its crystalline structure with more crystallinity compared to HA which is shown in figure 2(a). As the structure becomes more amorphous, its stability in biological pH decreases, therefore, bioactivity increases. Annealing of the bovine bone higher than 600°C–700°C removes its organic phase like protein and fat and just its mineral phase (Calcium phosphate) remains at higher than this range (see figure 2(c)). Based on the literature [18, 23], the XRD analysis of BP prepared by thermal decomposition of bovine bone at 800°C, containing hydroxyapatite phase, and its crystallinity and sharpness of peaks increased by increasing the temperature. During the thermal decomposition, at a temperature above 1000°C, some other phases of calcium phosphate such as beta Three
Calcium Phosphate ($\beta$-TCP, with the higher bioactivity in body environment in comparison with BP) may crystalize [23].

In order to investigate the powder morphology and size, SEM analysis has been used. The powders particle size of HA, BAG, and BP were measured 126.15 nm, 95.74 nm, and 417 nm respectively by an average of 15–20 particles using Digimizer software and SEM images representing in figure 3. It seems that BP particle size prepared by thermal decomposition has a larger particle size compared to HA and BAG synthesized by the sol-gel method. All the powders exhibit nearly similar morphology composed of polygonal and spheroidal shape. Since there is a tendency to agglomeration for experimented powders, it results in the formation of localized nanosize particle clusters with the highest amount in BP with a cluster size of more than one micron. BAG powder has the most porosity in comparison to the others and their pores are interconnected, despite the highly dense BP powder. The time and speed of the ball milling procedure are the important parameters in the particle size and agglomeration [24]. Nasiri-Tabrizi et al [25] investigated the effect of ball milling parameters on the particle size of hydroxyapatite extracted from the heat treated bovine bone. According to their results, the particle average size reaches to 40 nm and 34 nm after 40h and 80h of milling under the argon atmosphere and the speed of 600 rpm, respectively. It has been reported the particle agglomeration occurs after 80h milling under the argon atmosphere, consequently, there is optimum time and speed in order to reach the specific particle size especially for those with less than 100 nm during the ball milling procedure. By discarding the optimum time, the agglomeration will occur and the desirable particle size could not be achieved.

Figures 4–7 show cross-section micrographs of CP-Ti with and without powder. In each figure, grain refinement is obviously indicated. Moreover, the representative elemental mapping images for the processed Ti specimens are shown in figures 4–7 and accordingly, it can be seen the HA, BAG and BP particles are homogeneously distributed and no agglomeration of particles occurs in the SZ. Also, the predomination of the
Ca and P elements can be detected in the maps of Ti/HA and Ti/BP specimens (figures 2, 4). In addition to Ca and P elements, the EDS map for Ti/BAG (figure 5) indicates the presence of Si and Na elements as well. These elements, especially Si, have crucial importance to improve cell viability, proliferation, and cell adhesion ability of the implants [26]. According to the literature, by using the optimized parameters e.g. the rotational and travel speeds, the friction stir process may have a deagglomeration effect for second phase powders [27] which is in complete agreement with the EDS analysis presented in figures 4–7.

The surface roughness parameter (Ra) of experimented specimens is measured 20.6, 18.3, 22.2 and 14.1 μm for Ti/HA, Ti/BAG, Ti/BP and CPT processed specimens respectively by using the Profilometry test (see figure 8). Accordingly, the amount of ‘Ra’ increases by compositing the surface with ceramic powders and reaches to the maximum amount in the case of using the biggest particle size (BP, see figures 3 and 8).
3.2. Surface wettability and roughness

It has been reported the surface chemistry/topography and surface hydrophilic/hydrophobic properties can be determined by using a contact angle test [28]. As is shown in figure 9, the contact angle of titanium nanocomposites decreased by adding the biocompatible powder. The Θ angle was measured as 43.34°, 34.57°, 66.08°, 91.19° for Ti/HA, Ti/BAG, Ti/BP and CPT processed specimens, respectively. As is clear in figure 9, the FSP by alteration of the surface roughness, chemistry, and energy simultaneously (in the case of using the second phase powder) has a significant effect on the surface wettability. This is in complete agreement with the previous study which reported the surface wettability is affected by the surface roughness and the surface energy [29].

Based on the figure 9 and Wenzel equation [30], by increasing the surface roughness due to using the second phase articles, drop contact angle decreased and consequently the surface wettability increased. The highest hydrophilicity of Ti/BAG was related to its lowest crystallinity in comparison with the other powders (see figures 2 and 9). The same results have been reported in by Luz et al [31]. The surface becomes more hydrophilic in the case of HA as a modifying powder with more amorphous structure even in comparison with another used calcium phosphate powder (BP) with approximately similar ‘Ra’. According to the previous investigation, the rougher surfaces have more potential for initial cell adhesion [32]. On the other hand, the initial osteoblastic cells attachment and the protein adsorption may be affected by the surface wettability parameter [33, 34].

In order to investigate the surface morphology of titanium modified by friction stir process, SEM analyze has been utilized and in the case of untreated specimens before and after 14 days’ immersion test in SBF and the results have been depicted in figures 10 and 11, respectively.

3.3. Apatite characterization

Figure 11 represents the surface morphologies of processed CP titanium and corresponding EDS local elemental maps after 14 days soaking in simulated body fluid. According to figure 11, by the presence of the calcium phosphate particles and their clusters, the bone-like apatite nucleate and grow on the surface of treated specimens. The agglomeration of calcium and phosphate particles can be detected in higher magnification (shown by yellow arrows in figure 11). The formation of the apatite layer is detectable and clearly obvious on all the surfaces of specimens (figure 11) in comparison with the composite surfaces before immersion is shown in figure 10. In the case of two experimented calcium phosphates (HA and BP), it seems that the higher amorphous structure results in the higher dissolution (see figures 2 and 11(a), (e)). Also, as is shown in figures 11(c), (d), the
existence of the apatite layer is obvious on the surface of the Ti/BAG and EDS map shows additional elements like Si on the surface which may indicate the presence of silanol group. As a result of the condensation of silanol functional groups (Si-OH), the silica gel forms and provides the most preferable site for apatite nucleation [35]. Since the apatite layer is the most appropriate substrate for cell movement and protein adsorption, using BAG may result in faster bone bonding and bone tissue ingrowth [36, 37]. The existence of the TiO₂ phase which is formed during high-temperature FSP of CPT could be the preferable site for the apatite nucleation by the formation of the Ti-OH functional group in the body environment [38]. This fact has been proven by detecting the sign of the Ca and P elements distributed on the surface of titanium in the EDS map (see Fig. 11g). Hsu et al.
[39] reported no apatite formation on the surface of un-anodized titanium after 14 days immersion in SBF. While, after 14 days immersion of anodized pure titanium in SBF, the formation of an ultra-condensed apatite layer led to form micro-cracks in the Ca-P layer. It worth to mention no micro-crack formation in the Ca-P layer.

Figure 11. SEM images and EDS analyses of the biomimetic apatite layer on the different titanium nano-composites surface after soaking in simulated body fluid (SBF) for 14 days at 37°C (a,b) Ti/HA (c,d) Ti/BAG (e,f) Ti/BP (g,h) Ti without powder.
has been detected in the present work after 14 days of immersion while the apatite layer covered the entire titanium surfaces.

3.4. MTT assay- direct test (cell viability and proliferation)
For the time which is mentioned above, the MTT graph is illustrated in figure 12 and it can be observed that Ti/BAG has the highest cellular viability (95%) which is the evidence of its superior biocompatibility. It has been reported by alkalization of the medium as a result of the dissolution product from BAG degradation (especially Si ions) as well as the stimulation of extracellular channels in osteoblasts for Ca ions releasing, the proliferation may improve [40]. Although, the Ti and Ti/BP specimens have shown acceptable cellular viability (80% and 87%, respectively), the lowest viability percentage of Ti/HA is due to the cytotoxicity of this specimen. Cell death occurs when the pH of the medium becomes lower or higher than the standard value (pH = 7.4) [41]. During the over releasing of the ions like Ca and P from HA particles degradation, the cell death may occur as a result of deviation of pH from the standard value (pH = 7.4). On the other hand, the same powder volume has been used in the case of each experimented specimens. Therefore, the only variable experiment parameter was the powder density which could affect the concentration of the apatite layer contained Ca and P ions. Moreover, if the cell-extracellular Ca$^{2+}$ concentration unbalance by exchanging excessive Ca$^{2+}$, the cell membranes collapse and cause lower cell viability, thus impairing the cell homeostasis.

For example, Motskin et al [43] reported the most cytotoxicity for the gel method. Since the cytotoxicity is related to the particle uptake by cells, the main reason for the cell cytotoxicity could be the cellular particle load [44]. On the other hand, the uptake of the particles by cells is controlled by the particles parameter such as shape, charge, size and surface area [43, 45]. For instance, the rod shape and the positively charged particles have a lower uptake rate compared to the spherical and negatively charged ones [43].

3.5. Cell adhesion and morphology
The initial phase formed during superficial interaction has a crucial role in cell survival and its growth behavior. The SEM micrographs presented in figure 13 illustrate the MSCs different cell morphologies after 4 days of cell culturing on the different treated specimens. As is shown in figure 13, the cells are very well-attached on the treated surfaces except for Ti/HA specimen due to the cytotoxicity effect of the Ti/HA surface (as well discussed in the previous section). The flat, multipolar cells with fibrillar extensions and spindle-like morphology detected on all treated specimens (except for Ti/HA, see figures 13(c), (e), (g)) have been formed as a result of the surface chemistry alteration and the surface roughness produced by FSP. In addition, the cells on Ti/BP is highly stretched (figures 13(e), (f)). It can be seen in figure 13 that the polygonal morphology of MSCs has been detected even in much higher roughnesses which is in the agreement with the previous report that cell adhesion occurred on substrates with high roughnesses [32]. The Mesenchymal stem cells for all the specimens show the acceptable proliferation and cuboidal with dendritic extension morphology after friction stir process except for Ti/HA figure 13. In the case of these three specimens (BP, BAG, and Ti in figures 13(e), (c), (g)), the cells show a high tendency for cross-linking to their adjacent cells. Whereas the surface roughness is high in the case of Ti/HA in figures 13(a), (b), the rounded cells shape, inappropriate adhesion and lack of joining tendency are as a result of
the cell death due to its cytotoxicity (see figure 12) or low cell activity; therefore, it means the cells are not able to spread on the surface with polygonal morphologies. The superficial micro-cracks are detected alongside the cell body (see the yellow arrows in figures 13(d), (f)), these micro-cracks will cover later during cell attachment. The reason for such a good cell attachment detected in figures 13(g), (h) could be due to the oxidation of titanium and consequently the formation of the appropriate anchorage for the protein adsorption and the cell adhesion during the high-temperature process [46]. Moreover, Nayab et al [47] reported that the sufficient amount of Ca
can stimulate the integrin signaling pathway, therefore results in the better cell adhesion to the substrate. It can be concluded enhancing the ligand binding of the α5β1 integrin receptors and led to better cell adhesion to the substrate. It can be concluded enhancing the ligand binding of integrin receptors and led to better cell adhesion to the substrate.

4. Conclusion

In the present work, the surface of CP titanium was modified through friction stir processing (FSP) in order to fabricate the superficial titanium based nanocomposites. For this goal, bioactive glass 45S5 (BAG), hydroxyapatite (HA) and natural bone powder (BP) with different crystallinity were used as the second powder phase. According to the cross-sectional studies, grain refinement was obviously detected and the particles were homogeneously distributed in the stir zone with no sign of particle agglomeration. The surface treatment with biocompatible ceramic nanoparticles e.g. BAG enhanced the wettability of the pure titanium due to the hydrophilicity effect of these ceramics. Mineralization experiments revealed that an appetite-riched layer was successfully deposited throughout the surface of titanium nanocomposites after the immersion test in the simulated body fluid for 14 days. MTT assay revealed that the Ti/BAG had the highest cellular viability and proliferation and the low viability percentage of Ti/HA was due to the cytotoxicity of this specimen. Therefore, FSP was introduced as a capable technique to fabricate the titanium composites with desirable biocompatibility and appropriate mechanical properties.

ORCID iDs

Mohammad Javad Bathaei https://orcid.org/0000-0003-2281-2520
Jhamak Nourmohammadi https://orcid.org/0000-0002-9401-6470
Hamid Reza Abedi https://orcid.org/0000-0002-6921-1522

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