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TITLE PAGE

Title:

Effect of bioactive glass air-abrasion on the wettability and osteoblast proliferation on sandblasted and acid-etched titanium surfaces

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Bioactive glass air-abrasion enhances wettability and osteoblast proliferation on sandblasted and acid-etched titanium surfaces.

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Abstract

The aim of this study was to evaluate the hydrophilicity, surface free energy (SFE) and proliferation and viability of human osteoblast-like MC3T3-E1 cells on sandblasted and acid-etched (SA) titanium surfaces after air-abrasion with 45S5 bioactive glass (BAG), Zinc containing BAG or inert glass. SA discs were subjected to air-abrasion with 45S5 BAG, experimental BAG (Zn4) or inert glass. Water contact angles (CA) and SFE were evaluated. The surfaces were studied with preosteoblastic MC3T3-E1 cells. Air-abrasion with either glasses significantly enhanced the hydrophilicity and SFE of the SA discs. MC3T3-E1 cell number was higher for substrates air-abraded with Zn4 BAG and similar to borosilicate coverslips (CNTRL). Confocal laser scanning microscope images showed that MC3T3-E1 cells did not spread on the SA and BAG surfaces as they did on CNTRL. However, for 45S5 and Zn4 treated samples, cells spread most within 24 h time frame and changed their morphology to more spindle-like when cultured further. BAG and inert glass air-abrasion was shown to have a significant effect on the wettability and SFE of the surfaces under investigation. Osteoblast cell proliferation

was enhanced by 45S5 BAG and experimental BAG (Zn4) air-abrasion compared to inert glass air-abraded discs or SA discs without air-abrasion.

Keywords: Bioactive glass, sandblasting and acid-etching, titanium, osteoblast, contact angle.

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Introduction

Sandblasting and acid etching (SA) is the most commonly used surface modification for dental implants. Clinical studies have shown that the SA titanium surface is efficient in enhancing the osseointegration process (1, 2). However, the same surface also promotes biofilm formation once exposed to an oral environment, which may eventually lead to peri-implantitis and implant failure (3).

Peri-implantitis is a progressive and irreversible disease of implant-supporting hard and soft tissues. It is characterized by bone resorption, decreased osseointegration, increased pocket formation and bleeding on probing (4, 5). The ideal management of peri-implant diseases focuses on decontamination of implant surfaces and regeneration of lost tissues (6, 7). Several conventional treatment modalities, including curettes, ultrasonic scalers, and air powder abrasion systems, have been used for the detoxification and debridement of contaminated titanium surfaces. However, these conventional approaches are frequently insufficient to lead to the complete removal of bacterial biofilms from rough implant surfaces (8, 9)

Air-particle abrasion with a bioactive glass (BAG) has shown antimicrobial effect and suppressed bacterial biofilm formation on titanium surfaces *in vitro* (10). The composition of bioactive BAG is commonly based on SiO2-Na2O-P2O5-CaO (11). The antibacterial activity of BAG has predominantly resulting from its high pH, and osmotic effects caused by the non-physiological concentrations of different silicon species as well as sodium and calcium ions dissolved in the glass (12, 13). The incorporation of zinc oxide (ZnO) into the well-known bioactive glass 45S5, was found to be effective in controlling its chemical durability and bioactivity (14). Moreover, this could stimulate osteoblast proliferation and differentiation, thus improving the bond of implants with bone (15, 16). The slow release of zinc incorporated into an implanted material has been reported to stimulate bone formation around the implant (17).

Surface free energy (SFE) of implant surfaces is known to play an essential role in initial protein conditioning of the implant surface and subsequent cellular adhesion (18). SFE is divided into polar and non-polar components. Surfaces with high polar SFE components have low contact angles (CA) with polar liquids such as water (19), which leads to an increase in wettability. Increased wettability improves the interaction between the implant surface and the surrounding biological environment (20, 21). Surface hydrophilicity also determines the biocompatibility of biomaterials and is mostly dependent on surface energy (22). WALL *et al.* (23) demonstrated that osteoblast adhesion and proliferation are directly related to the surface microarchitecture and SFE. ERIKSSON *et al.* (24) showed that the rate and extent of bone formation are increased with increased implant surface hydrophilicity.

This study aimed to evaluate the hydrophilicity and SFE of SA titanium surfaces after air-particle abrasion with BAG, Zn containing BAG or inert glass. An additional aim was to study the attachment and proliferation of human osteoblast-like MC3T3-E1 cells on the same surfaces.

Materials and methods

Sandblasted and acid-etched (SA) surface preparation

Grade five cpTi square sheets of 200mm and a thickness of 1mm (Glocon, Vantaa, Finland) were cut into squares discs of 10 mm. Large grit aluminum oxide (Al_2O_3) particles 250-500μm (Edelkorund, Eisenbacher Dentalwwaren, Wörth am Main, Germany) were used for sandblasting the titanium discs utilizing a sandblaster (Renfert Basic ECO Sandblaster, Beckum, Germany) with an air pressure of 5 bar. Then, the discs were acid-etched in HCl (60%) and H_2SO_4 (70%) mixture for 1h in 60°C in a hot air oven (Binder MDL 115 Drying Oven, Tuttlingen, Germany). The SA discs were then thoroughly rinsed with deionized water in an ultrasonic bath (L&R Quantrex 90 Ultrasonic Cleaner, Kearny, New Jersey, USA) for 20 min to eliminate acid residues. The discs were placed in a dry hot air oven for 30 min at 50°C.

Abrasive glass preparation

The bioactive glasses (BAGs), Zn4 and 45S5, were melted in-house. Batches giving 300 g of glass were mixed with analytical grade chemicals Na_2CO_3, $CaHPO_4 \cdot 2H_2O$, ZnO (all from Sigma-Aldrich, st Louis, Missouri. USA), $CaCO_3$ (Fluka, Charlotte, North Carolina, USA), H_3BO_3 (Merck, Kenilworth, New Jersey, USA), and Belgian glass quality quartz sand. Nominal composition (mol.%) 45S5 and experimental BAGs is given in Table1. Network connectivity (NC) for the BAGs was calculated according to HILL & BRAUER (25). In Zn4, 4 mol% ZnO was substituted for 2 mol% of both SiO_2 and CaO, thus decreasing the calculated network connectivity from NC=2.12 for 45S5 to NC=1.94 for

Zn4. The oxide composition of the prepared bioactive glasses is presented in Table 1. The glasses were melted in an uncovered platinum crucible at 1360 °C for 3 h in air. Following casting, the obtained glass blocks were annealed at 520 °C for 1 h and then slowly cooled in the oven. The blocks were then crushed and re-melted to ensure homogeneity. Then, the blocks were crushed and sieved to give particles of the size range fractions 45-120µm and 300-500 µm. The particle morphology was examined with a scanning electron microscope (SEM, Leo Gemini 1530, Hamburg, Germany). Inert glass was commercial float glass.

Ion release measurement

The release of ions from the Zn4 and 45S5 BAGs into simulated body fluid (SBF) were measured in dynamic condition. The SBF solution was prepared according to the protocol suggested by KOKUBO & TAKADAMA (26). The release of the ions in dynamic conditions were measured using the experimental setup reported by FAGERLUND *et al.* (27). The glass particles (284.6 mg, 300-500 µm) were weighed into a reactor after which fresh SBF at 37 °C was continuously fed vertically from bottom to the top of the reactor. The concentrations of the ions released from the glass particles were measured on-line for the first 20 min using Inductively coupled plasma - optical emission spectrometry (ICP-OES) and after that for samples with a total volume 3 mL collected for selected time points up to 48 h.

Air-particle abrasion of SA titanium discs with BAGs and inert glass

The SA titanium discs were subjected to air particle abrasion with Zn4 and 45S5 BAGs or inert glass. An air-abrasive device (LM ProPower, Parainen, Finland) was used for the SA disc abrasion process

with glass particles as specified above. The air-abrasion procedure was performed for 20 sec, at 90° angle and 3mm distance using air pressure of 4 bar.

Surface roughness measurements

The surface roughness of SA titanium and all abraded discs were evaluated with a surface roughness tester (Leitz Wetzlar 601908, Wetzlar, Germany) and surface roughness values (RA) were calculated. Five measurements were recorded for each specimen.

Contact angle (CA) measurements

The surface wettabilities of SA titanium and all abraded discs were evaluated from contact angle measurements using the sessile drop method described by JONG *et al.* (28) with Theta Optical tensiometer (KSVCAM100 KSV, Instrument, Oulu, Finland). One hundred twenty images were captured in 20 sec for each drop deposited on the surface. In practice, a droplet is placed on the titanium substrate surface and the image of the droplet is recorded. The contact angle is then defined by fitting Young-Laplace equation around the droplet. Sessile drop method was combined with an axisymmetric drop shape analysis (ADSA) technique to provide both contact angles and surface tension based on the Young–Laplace equation.

Surface free energy (SFE) calculations

SFE of the substrates were calculated using the Owens-Wendt (OW) approach. In the OW method, the SFE (γ_S) of a solid material is a sum of a short-range polar and a long-range dispersion components (29). The polar component (γP) is a sum of hydrogen, acidic/basic and induction interactions, whereas the dispersion component (γd) defines the strength of intermolecular interactions named London forces. Distilled ultrapure water, Diiodomethane >99% purity and

Formamide pro analysis were used as a probe to calculate the surface free energy of the SA titanium and all abraded discs using the sessile drop method (KSVCAM100 KSV, Instrument, Oulu, Finland). The following equation is used:

$$1 + \cos\emptyset = 2(y_s^d)^{1/2}\left(\frac{\left(y_L^d\right)^{1/2}}{yL}\right) + 2(y_s^P)\left(\frac{(y_L^P)^{1/2}}{yL}\right)$$

Where $\varnothing$ is the contact angle between tested surface and standard liquid, y_s is the SFE of the surface and yL, the SFE of the liquid.

Scanning Electron microscopy

Titanium discs were carbon sputter coated using Emscope TB 500 Temcarb (Thermo VG, Waltham, Massachusetts, USA). SEM images and Energy-dispersive X-ray spectroscopy (EDS) analysis were performed using scanning electron microscopy (LEO Gemini 1530, Carl Zeiss, Hamburg, Germany). The X-ray detector (EDS) by Thermo Scientific, Madison, Wisconsin, (USA). Confocal laser scanning microscope Zeiss LSM 750 Meta (Oberkochen, Germany) was used for acquiring the osteoblast confocal images.

Osteoblast cell study

Cell culture

Preosteoblastic MC3TC-E1 cells (subclone IV, ATCC. LCG Promochem, Manassas, Virginia) were cultured in α-MEM (Corning, New York, USA) including 10% FBS (Biowest, Nuaillé, France), 1% penicillin/streptomycin and 1% l-Glutamine (Gibco, Waltham, Massachusetts, USA). The cells were cultured in a humidified atmosphere of 5% CO_2 at 37◦C and passaged every other day before culturing onto test materials. Before the cell studies, titanium discs were placed in 24-well Tissue Culture Polystyrene (TCPS) plates, immersed in 70% ethanol for 1 h and washed with 1× PBS for 3 × 15 min and subsequently immersed in PBS overnight. Finally, the cells were trypsinized with 0.5%

trypsin-EDTA (Gibco, Waltham, Massachusetts, USA) and seeded onto the titanium discs or control cover glasses (Ctrl) at a density of 5×10^3 cells per well in 1 ml medium.

Proliferation and viability

Cell proliferation and viability were measured using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay at 1, 3 and 6 d of cell culture. The cell culture medium was completely replaced with new medium containing MTT (0.1 mg/ml medium) and the SA and abraded discs, and smooth borosilicate coverslip (controls) were incubated at 37˚C for 3 h. Unincorporated dye was removed, dimethyl-sulfoxide (DMSO) was added, and absorbance was quantified by a plate reader (Victor 2, PerkinElmer Life Science/Wallac, Turku, Finland) at 550 nm. The result was background-corrected at an absorbance of 650 nm. The results were expressed as the average absorbance values of four replicates.

Fixation, fluorescence microscopy

Cells were cultured on all samples for 2, 24 and 48 h. After culture, the cells were fixed with a 3% paraformaldehyde (PFA) solution in PBS containing 2% saccharose for 10 min at room temperature (RT) and washed twice with PBS. After fixation, the cells were permeabilized with 0.1% (v/v) TritonX-100 in PBS (10 min on ice) and blocked with 0.2% (v/v) bovine serum albumin (BSA, 30 min in RT). After extensive washing with PBS, the actin cytoskeleton of the cells was stained with 1:200 diluted TRITC-phalloidin (Sigma) for 3h at 37◦C. After several washes with PBS, cell nuclei were stained with 1:800 diluted Hoechst 33258 (Sigma) for 10 min at RT. The cells were imaged using a confocal laser scanning microscope Zeiss LSM 750 Meta (Oberkochen, Germany) with a 63x objective (NA 1.32).

FE-SEM

For SEM analysis the cells were rinsed twice with PBS and fixed in 2.5% glutaraldehyde for 15 min in 0.1 M phosphate buffer, dehydrated in graded ethanol series and dried using critical point dryer

(K850, Quorum Technologies, East Sussex, UK). Dried samples were attached to aluminum specimen mount using double sided carbon tape and coated with 5 nm of platinum (Q150T ES, Quorum Technologies, UK). Samples were examined in Σigma HD VP SEM (Carl Zeiss Microscopy GmbH, Oberkochen, Germany).

Image analysis

The cell number, cell surface area and cell shape were analyzed with Image-J 1.52 software (Fiji collection) from spatially calibrated FESEM images at 14x magnification (6144x4608 pixels). Random regions of interest (ROI) of 5.55mm^2 were point counted with cell counter plugin. The cell area and aspect ratio were analyzed with shape descriptors plugin after manually tracing the cell perimeters.

Statistical analysis

The distributions of the study variables were studied and described. Measurements were reported as means and standard deviations (SD). The differences between the experimental treatments were evaluated using one-way analysis of variance (ANOVA) followed by post-hoc Tukey HDS tests at an alpha level of 5%. The results were determined to be significant at $p<0.05$. If normality assumptions were violated the logarithmic transformation was adopted, and the testing was repeated. Statistical analyses were performed using the SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA).

Results

Ion release

After the initial burst ceasing at around 5 min, the ion release measured on-line during the next 15 min of SBF flow through the particles was rather constant for Si and Ca. For 45S5 BAG, the Si-ion

concentration was at around 40 mg/L while 7 mg/L was measured for Zn4 (Fig 1a). The Ca-ion release between 5 and 15 min was higher for 45S5 BAG, around 120 mg/L, and for Zn4 BAG, the corresponding concentration was 80 mg/L (Fig. 1b). The concentration of P gradually increased and was at 20 min around 35 mg/L for 45S5 BAG and 25 mg/L for Zn4 BAG (Fig. 1c). The concentration of Zn-ions released from Zn4 was low but gradually increased (Fig. 1d).

SEM image observations and EDS analysis

The SA disc surfaces before and after air-abrasion are shown in Fig. 2. SEM images showed that sandblasting and acid-etching of the titanium surface produced apparent surface roughness at macro and nano scales (Fig. 2a). Following air-particle abrasion the abrasive glass particles smoothened the SA surface topography (Fig. 2b-d). EDS analysis of these surfaces showed that elements of BAG or inert glass used for air-abrasion remain trapped on the SA surface (Fig.3).

Surface roughness and contact angle measurements

The results of the surface roughness test are shown in table 2. The surface roughness values were significantly higher for SA surface before BAG air-abrasion, [$F(3, 16) = 386.1$, $p<0.001$]. No differences in surface roughness among BAG/inert abraded surfaces.

The surface contact angle values are shown in Fig 4. Non-abraded SA discs were hydrophopic with highest mean water contact angle value (113.4°). The air-abrasion of SA discs made the surfaces hydrophilic, [$F(3, 20) = 2963$, $p<0.001$]. The Zn4 BAG air-abraded titanium discs had the lowest mean water contact angle (10.5°) followed by 45S5 BAG abraded titanium discs (14.7°) and inert glass abraded discs (19.3°).

Surface free energy calculations

The SFE result is shown in Fig. 5. The air-abraded titanium discs group showed significantly higher total (γ^{tot}) SFE [$F(3, 20) = 1931.2$, $p<0.001$] and higher polar (γ^{p}) SFE [$F(3, 20) = 3676.7$, $p<0.001$]

components compared with SA discs. No significance differences among the groups in dispersive (γ^d) SFE component [$F(3, 20) = 1$, $p = 0.413$] was found.

Cell proliferation and viability

Cell proliferation rates are shown in Fig. 6. The MC3T3-E1 cells proliferated on all samples. The proliferation rate was significantly higher at d1 for 45S5 and Zn4 BAG air-abraded titanium discs compared to titanium discs abraded with inert glass or SA discs [$F(3, 12) = 44.85$, $p<0.001$]. No significant differences could be observed among the groups when MC3T3-E1 cell culturing was extended to 3 or 6 d.

Visualization of MC3T3-E1 preosteoblasts on titanium surfaces

Confocal laser scanning microscope images and Field Emission Scanning Electron Microscope (FE-SEM) images showed that MC3T3-E1 preosteoblasts spread well on smooth borosilicate coverslip. However, these cells did not spread on SA but attached firmly to the rough titanium surface. The cells spread most at the 24 h time point on all samples and changed their morphology to spindle-like when cultured further (Fig. 7 and 8). Fig. 9 shows that cells were evenly distributed on the whole surface. The cell number count result per 5.55mm^2 is shown in Fig. 10a. Zn4 and CTRL demonstrated higher cell count number in all time points compared to 45S5/inert surfaces or SA surfaces. Cell surface area was similar for all titanium samples (Fig. 10b). 45S5 and Zn4 BAG abraded samples showed higher cell shape aspect ratio compared to SA or inert glass abraded samples (Fig 10C). These results indicate that the cells tolerated well the air-abraded SA surfaces compared to non-abraded SA surfaces.

Discussion

The results of this study show that BAG or inert glass air-abrasion of SA discs makes rough titanium surfaces hydrophilic and increases their SFE values. Our study also demonstrated that osteoblast cell proliferation was considerably higher on SA discs subjected to BAG air-abrasion compared to inert glass air-abraded or non-abraded SA discs.

Generally, the contact angle (CA) can range from 0° to 180°, indicating that the wetting liquid drop is spreading or beading, respectively. Water CAs less than 90° designate surfaces as hydrophilic, while CAs very close to 0° identify surfaces as super-hydrophilic. Surfaces with CAs above 90° are considered hydrophobic, and surfaces with CAs above 150° are often termed super-hydrophobic. In our study, the mean water CA of 113° was obtained for the SA surface. This value is in agreement with earlier experimental studies showing strong hydrophobicity for SA surface produced by a similar method used in our study (29, 30). SA surfaces contain two roughness scales; microroughness which originates from the sandblasting process and nanoscale roughness created by the acid-etching process. It is possible that the microroughness on the titanium surface can initially be filled with water while the smaller etched pores cannot, thus explaining that the surface is strongly hydrophobic. Air-abrasion of SA surfaces with either BAG or inert glass changed the CA to more hydrophilic compared to un-abraded SA discs. The change in the surface wetting behavior could be explained by the fact that particles used for air-abrasion got trapped within the pores on roughened SA surfaces leading to smoother surface microtopography as the Ra roughness values were significantly lower for BAG/inert air-abraded samples when compared with SA surface without abrasion.

SFE reflects the affinity of the surface to other materials; the higher the SFE the more energy is gained upon bringing this surface into contact with other materials (31). Our results showed that BAG or inert glass air-abrasion caused a significant elevation in γ^{tot} and γ^{p} component compared to

non-abraded SA surfaces. These differences cannot be explained by the dispersive component of SFE, because there was no significant difference in γ^{d} among all tested surfaces.

MC3T3-E1 cells were chosen for this study owing to their high levels of osteogenic differentiation and similar behavior to primary osteoblasts, which makes them widely used cells for *in vitro* experiments (32). Our results show that high SFE is essential but not the only variable to cause a marked increase in osteoblast response. Our observations are consistent with the previous finding reported by ZHAO *et al.* (18). Although inert and BAG air-abraded SA discs showed similar SFE values, BAG air-abraded SA discs showed significantly higher osteoblast viability compared to inert glass air-abraded discs. Ion release from BAG particles on the abraded surface seems to play an essential role in osteoblast proliferation especially in the first d of culture.

BAG 45S5 was chosen because it is often considered as gold standard for bioactive glasses. its ionic dissolution products are known to induce osteoblast proliferation (33). In our experiment, the concentration of ions in SBF were measured at several time points during feeding fresh solution through the glass particle for up to 48 h. As the flow rate was 0.2 mL/min, the total volume of solution consumed during the experiment was high, around 570 mL for each batch of 280 mg glass particles. Accordingly, the ion concentrations clearly decreased with time simultaneously when the amount of the glasses decreased. Figure 1 (a-c) give the concentrations of the Si, Ca and P species released from the glasses during the experiments. The concentration of Zn^{2+} was at a low level over the test time of 48 h, at around 0.2 mg/L.

Hench (2009) reported that the ionic dissolution products released from BAG 45S5 were biologically active soluble Si and Ca ions. These ions produced osteoblast cell stimulation at a particular concentration range of 15-30 ppm Si and 60-90 ppm ca. (34). The ionic dissolution products of BAG have been reported to increase insulin-like growth factor II (IGF-II). IGF-II is an anabolic peptide of

the insulin family and comprises the most abundant growth factors in the bone. BAG ionic dissolution increases IGF-II availability in osteoblasts by inducing the transcription of the growth factor and its carrier protein and by balancing the dissociation of this factor from binding protein (35). Xynos et al. (2000) reported that the unbound IGF-II is likely to be responsible for the increase in osteoblast cell proliferation (36).

The experimental glass Zn4 was based on partial molar substitution of ZnO (4 mol-%) for both SiO_2 (2 mol-%) and CaO (2 mol-%) in 45S5 BAG. ZnO is a network modifying oxide known to increase the chemical stability of bioactive glasses. ZnO has been found to modify the silicate network structure as the oxide tends to decrease the degree of network connectivity by replacing the binding oxygen which forms the connection between two SiO4 tetrahedra by non-bridging oxygen. (37). In addition, Zn^{+2} is known to play an essential role in stimulating osteoblast cell proliferation *in vitro* (38). Zinc has been reported to stimulated cell proliferation and differentiation, as well as protein synthesis in osteoblastic MC3T3-E1 cells (39). The cellular mechanism of how zinc stimulates osteoblast cells has not been fully understood. Zinc supplementation in cell culture medium of MC3T3-E1 murine osteoblasts has been found to enhance mineralization and osteocalcin mRNA expression levels of these cells (40).

FE-SEM and confocal laser scanning microscope images of cell morphology identified differences in cell shape between titanium discs. Cell surface area was similar with all the titanium surfaces and much smaller than on CNTRL (Fig. 9 B). However, the aspect ratio increased in time and the cells were most spindle shape on 45S5 and Zn4 samples (Fig.9C). The air-abrasion process decreased the roughness (Ra value) of the SA surface in some extent but that did not influence the cell shape although it is known that osteoblasts spread more on the smooth surface compared to a rough surface (18). Based on the results of this study BAG particle air-abrasion of SA surface leads to

increase in osteoblast viability mainly due to improved surface wettability and an increase in the SFE of SA discs. Better cell response can also be a result of ion release from the BAG particles trapped on the abraded surfaces. However, it is important to bear in mind that an *in vitro* conducted experiment has limitations, and the true potential of BAG particles air-abrasion treatment of peri-implantitis involved implant surfaces needs to be evaluated in clinical settings.

Bioactive glass and inert glass air-abrasion enhances the wettability and surface free energy of sandblasted and acid etched titanium surfaces. The proliferation of pre-osteoblastic MC3T3-E1 cells increases after 45S5 or Zn4 BAG air-abrasion. MC3T3-E1 favors the bioactive glass or inert glass air-abraded sandblasted and acid etched surfaces compared to non-abraded surfaces.

Conflict of interest:

Authors declare that this paper is original and free of any conflict of interest

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Table Heading:

Table 1. Nominal composition (mol.%) 45S5 and experimental zinc oxide containing BAGs. Network connectivity (NC) for the BAGs was calculated according to HILL & BRAUER (25).

Figure Legends:

Figure 1. Concentration of Si-species (a), Ca-ions (b) and P-species (c) in SBF fed through the particles as a function of time. Initial release of Zn^{2+} from Zn4 into continuous flow of fresh SBF (0.2 mL/min) (c).

Figure 2. SEM image of sandblasted and acid-etched (SA) titanium surfaces at 1KX magnification (a). After air-abrasion with 45S5 bioactive glass (b), Zn4 bioactive glass (c), and inert glass (d).

Figure 3. EDS analysis showing surface chemical analysis of sandblasted and acid-etched (SA) discs (a), after air-abrasion with 45S5 bioactive glass (b), Zn4 bioactive glass (c) and inert glass (d).

Figure 4. Mean and standard deviation of water contact angle determination on sandblasted and acid-etched (SA) titanium discs and after 45S5 bioactive glass, Zn4 bioactive glass and inert glass air-abrasion, ***$p<0.001$.

Figure 5. Mean and standard deviation of total (OW.Tot), dispersive (OW.D) and polar (OW.P) surface free energy determination on sandblasted and acid-etched (SA) titanium discs and after 45S5 bioactive glass, Zn4 bioactive glass and inert glass air-abrasion, ***$p<0.001$.

Figure 6. Osteoblast cell viability and proliferation assessed by MTT assay for 1 d, 3 d and 6 d. Absorbance was measured in replicates of four at (650 nm) absorbance, ***$p<0.001$.

Figure 7. MC3T3-E1 osteoblasts cultured on sandblasted and acid-etched titanium discs or glass coverslips (CNTRL) for 2 h, 24 h and 48 h. The cells were stained with fluorescently labelled phalloidin for actin and Hoechst 33258 for nuclei. The confocal microscope images were acquired with 63x objective (frame size 143 x 143 µm). Scale bar 10µm.

Figure 8. MC3T3-E1 osteoblasts cultured on sandblasted and acid-etched titanium discs or glass coverslips (CNTRL) for 2 h, 24 h and 48 h. The FE-SEM images were acquired with 500x magnification. Scale bar 10µm.

Figure 9. MC3T3-E1 osteoblasts cultured on sandblasted and acid-etched titanium discs or glass coverslips (CNTRL) for 2 h, 24 h and 48 h. The FE-SEM images were acquired with 14x magnification. These images represent random ROIs. Scale bar 200µm.

Figure 10. Cell number / 5.55mm^2 ROI is an average of 3 similar randomly paced ROIS over the 100 mm^2 samples at various time points (a). Average cell surface area is expressed in µm mm^2 (b). The cell shape is described as an aspect ratio, which is calculated as maximal cell diameter per minimal cell diameter (c).