Resin and osteoblastic adhesion on zirconia and titanium implant materials blasted with various grits

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Abstract

The aim of this study was to evaluate the resin and initial osteoblastic adhesion of zirconia and titanium implant surfaces grit-blasted with four different sands, namely silica-coated alumina, alumina, silicon carbide and boron carbide.

Titanium and sintered zirconia specimens were polished and grit-blasted with one of the following grits: silica-coated alumina, alumina, silicon carbide and boron carbide. Two study groups were prepared. For the first group, a silane coupling agent and a resin was applied on grit-blasted sample surfaces (n=8), and adhesive strengths of the dental resins to these specimens were evaluated under shear mode in three storage conditions: dry, 24h water aging and thermo-cycled for 6000 cycles. The results were analyzed by using two-way ANOVA test with $\alpha=0.05$. For the second group, the specimens were immersed in a cell line medium (MC3T3-E1) and the attachment was observed under a confocal microscope after 24 hours. The attached cells were fixed and viewed under a SEM to observe the cell morphology.

It was found that the surface topography and chemical composition of zirconia and titanium were changed after grit-blasting with four different grits. The specimens grit-blasted with silica-coated alumina or alumina exhibited a significantly higher mean resin adhesive strengths ($p < 0.05$) than other two grits. In addition, SEM and confocal microscopy confirmed the specimens grit-blasted with alumina powder showed the maximum osteoblastic attachment, and revealed the cell morphology.

With the limitation of the laboratory study, alumina deemed to be the best grit-blasting material to achieve satisfactory both osteoblastic cell and resin adhesions for
titanium and zirconia implant materials.
Introduction

The surface of dental implants has been long under intensive research and has been identified as one of the areas providing the intimate contact between bone and tissue needed for healing and osseointegration. Titanium oxides (TiO₂), a complex surface layer which forms spontaneously on titanium upon exposure to the atmosphere, is the one which promotes osseointegration as opposed to the pure titanium surface (Carlsson et al. 1986). Hence, it is evident that the surface plays a vital role in permitting osseointegration leading to the stability of a dental implant. Following Brånemark’s theory of osseointegration, another breakthrough in the field of implant dentistry came with the introduction of textured dental implants and the importance of surface area (Gelb et al. 2013).

Many histological studies have demonstrated that surface roughness on implants had led to a higher bone-to-implant contact in the early healing phase and may achieve better survival rates in complicated cases when compared to machined implants (Buser et al. 1991; Ericsson et al. 1994; Gelb et al. 2013; Testori et al. 2002; Thomas & Cook 1985). Grit-blasting is one of the most effective and commonly employed modes of substantial surface modification. In general, measuring the adhesive strength between resin and the substrate material by shear (previously also known as “shear bond strength”) is one way to assess the adhesive stability and success for a restorative appliance in laboratory. Thus, it is evident that surface texturizing via grit-blasting can be advantageous for an implant on the aspect of an increased rate of osseointegration - as well as an increase in the adhesive strength in laboratory conditions.

The surface roughness and chemical composition depend on the type of grit-blasting material used and the particle size of the grits and blasting pressure. Various studies (Guo et
al. 2010; Matinlinna et al. 2013; Spohr et al. 2008) have proved and confirmed the effectiveness of grit-blasting and silanization (when first silica-coated) to improve the resin adhesive strength in various dental applications, e.g. Titanium, porcelain, zirconia, and even dental amalgam (Jin et al. 2015). This said, SiO$_2$, Al$_2$O$_3$ grits or Rocatec™ system (employing silica-coated alumina particles) have been some of the most successful surface pretreatment measures tested (Lung et al. 2012; Lung & Matinlinna 2012; Matinlinna & Vallittu 2007). In particular to Zirconia, recent studies (Liu et al. 2014; Liu et al. 2015; Sanli et al. 2015) has revealed that grit-blasting might provide a better initial resin adhesion strength than other treatment methods such as HF etching and glazing, but the adhesive strength is also affected by the stabilizing agent to the zirconia (Camposilvan et al. 2015a; Camposilvan et al. 2015b). In addition, other grits such as boron carbides (B$_4$C) and silicon carbide (SiC) are also commonly used in dentistry as abrasives on dental burs, due to their high Mohs hardness (>9). Thus, these carbides might be useful for roughening the sintered zirconia, which has a Mohs hardness of >7.

It is noteworthy that surface roughness has also been proven to be an optimal environment for osteoblastic cell attachment (Bowers et al. 1991; Buser et al. 1991; Degasne et al. 1999; Lincks et al. 1998; Martin et al. 1995). Surface roughness has been suggested to influence cell response and a rough micro topography was the best design for an orthopedic implant on pure titanium surface (Lincks et al. 1998). An earlier report (Bowers et al. 1991) also showed a significantly higher level of cellular osteoblastic adhesion was found on rough, grit-blasted surfaces with irregular morphologies while compared to smoother surfaces. However, such a rough surface might also promote other microbes to growth. Villard et al. has recently found that surface pretreatment by grit-blasting together with silane application could effectively improve some biological condition, such as inhibition of *C. albican* biofilm
formation (Villard et al. 2014) on titanium surface. Therefore, proper roughness and surface chemistry are the two most crucial points for microbes to either promote or inhibit the growth on implant materials.

Recently, single piece dental implants have been launched in the dental market. In fact, such a kind of implants was claimed to have simpler and convenient restoration procedures due to the abutment and fixture (i.e. screw part) was prepared as one single piece. Indeed, despite limited literatures are available for the long-term clinical performance, wear (Klotz et al. 2011; Stimmelmayr et al. 2012) and fatigue (Stimmelmayr et al. 2013) has been proven at the interfaces between the abutments and fixtures in the commonly used two pieces dental implant. Thus, the implant components could be loosened, and might subsequently fracture into pieces and possibly increase the risk of releasing titanium or zirconia debris (Klotz et al. 2011). This said, single piece dental implants would overcome these problems and could be a good option for cases, e.g. single tooth restoration, and could be used for immediate loading (Ghaleh Golab et al. 2015; Lauritano et al. 2014) with a properly trained dentist. Biologic and prosthetic complications were reported (Mangano et al. 2015), possibly due to the surface design in the single piece implant usually appeared to be smooth in the upper abutment part and rough in the lower fixture part. Clearly, the smooth abutment part might not be able to provide sufficient micromechanical retention for resin adhesion. As the prosthetic treatment with implants has to be completed with a dental crown, say, acrylic resin crowns are commonly integrated with single piece implants for immediate loading, thus the resin adhesion to zirconia and titanium, depending on grit-blasting techniques and materials has to be investigated.

For the fixture part, improper roughness might affect the cell growth and cell
movement (Abraham 2014), and various abrasives could also alter surface texture and chemistry such that the microbial adhesion quality on implant surface were affected (Duarte et al. 2009). To the best of the authors’ knowledge, there is no report on a single grit-blasted implant surface that could satisfy both resin and osteoblastic adhesions. Therefore, a suitable implant surface profile blasted by grits is essential and promising for the adhesions, as well as simplifying the manufacturing procedure.

There were two aims for this laboratory study: firstly, to investigate the resin adhesion; and secondly, initial osteoblastic cell attachment to zirconia and titanium surfaces when they were grit-blasted with four different grits. The hypothesis was that no significant difference were shown for both resin and osteoblasts adhesion on titanium and zirconia surfaces blasted by the four grits.

Materials and Methods

Specimen preparation

Thirty-six zirconia blocks (3M ESPE, Lava™ Frame, St. Paul, USA) of 9 mm × 12 mm × 3 mm were cut and sintered according to the manufacturer’s instructions. Also, twelve titanium (c.p. 2) sheets (Permascand, Ljungaverk, Sweden) with the size 20 mm × 40 mm × 1 mm (for resin adhesion test) and twenty-four coupons (for osteoblastic cell attachment test) 10 mm × 10 mm × 1 mm were used.

A total of four grit-blasting powders were used: 1. silica-coated alumina (110 μm; Rocatec™ Pre, 3M ESPE, St. Paul, MN, USA); 2. Alumina (110μm, 3M ESPE); 3. Silicon carbide (SiC) (105 μm-125μm, Hing Lung Engineering, Hong Kong); 4. Boron carbide (B₄C) (105 μm -125 μm, Hing Lung Engineering, Hong Kong). Each of the above described zirconia and titanium sample was firstly polished and cleansed ultrasonically in 70% ethanol.
for 10 minutes. Then, the samples were allowed to air dry in laboratory at room temperature. Next, the zirconia and titanium samples were randomly divided into 4 different study groups per grit-blasting material and they were grit-blasted using the Shofu Pen-Blaster (Shofu Dental MFC., Kyoto, Japan) as described in the literature (Matinlinna et al. 2006).

For the surface roughness ($R_a$), 4 zirconia and 4 titanium samples were randomly selected and measured using a profilometer (Surtronic 3+, Taylor-Hudson Limited, Leicester, England) individually at 3 different points. The control cut-off value was set at 5.9 $\mu$m as predetermined by the manufacturer. After testing, the samples were mounted on aluminium stubs and further characterized with SEM and EDX (SU1510, Hitachi High-Technologies Corporation, Tokyo, Japan) using the acceleration voltage of 12.00kV in high vacuum mode.

**Resin adhesion strength test**

For the resin adhesion strength (RAS) test, a dental silane primer (RelyX™ Ceramic Primer, 3M ESPE, St. Paul, MN, USA) was applied onto the grit-blasted titanium and zirconia surfaces with one coat by a fine brush provided by the manufacturer and allowed to react for 5 min at room temperature (Lung et al. 2012). Next, the resin cement (RelyX Unicem, 3M ESPE, Germany) was activated as instructed and packed into a custom-made polyethylene mould (diameter 3.6 mm, height 4.0 mm) and light-cured using a light curing unit (Elipar™ 2500, 3M, Seefeld, Germany) for 20 s from the top and 20 s from the lateral direction. Two resin stubs were bonded per one zirconia block and 8 resin stubs per titanium sheet.

The samples (n=8) were next randomly divided into three subgroups and stored in:

1. Dry condition: a dry desiccator for 24 hours,
(2) Wet condition: in deionized water at 37°C in an incubator for 30 days, and
(3) Thermo-cycled condition: thermo-cycling for 6000 cycles with a 20 second immersion time between two deionized water baths of temperatures 5.0 ± 0.5°C and 55.0 ± 0.5°C according to ISO 10477.

Then, RAS under shear mode was tested using a universal testing machine (ElectroPuls™ E3000, Instron, USA) at a crosshead speed of 1 mm/min with a maximum force of 500 N/mm², and obtained values were further calculation according to the formula:

\[ \text{RAS} = \frac{\text{load at fracture}}{\text{bonding area of resin stub}} \]

Following the shear bond strength test, the failure mode of each resin stub was analyzed with the naked eye and categorized as adhesive, cohesive or mixed failures. For those samples with fracture site consisting of less than 1/3 remaining resin were categorized as adhesive failure, 1/3 to 2/3 of remaining resin as mixed failure, and more than 2/3 remaining resin as cohesive failure (Matinlinna & Lassila 2010).

**Osteoblastic cell culture and seeding**

For the initial osteoblastic cell attachment, one specimen each of zirconia and titanium per grit-blasting material (12 grit-blasted zirconia blocks and titanium coupons, respectively) was used and the experiment was triplicated. They were lasted according to the same protocol as described above. Possible differences between the groups, concerning the osteoblastic cell attachment to the surface of the material were observed using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

The selected cell line, MC3T3-E1 was cultured using Minimum Essential Medium (MEM) α (Gibco® MEM α) supplemented with 10% fetal bovine serum (FBS) (Gibco®
sера). The MEM α was stored in a sodium bicarbonate buffer system (2.2 g/L), with 5-10% CO₂ and 37°C. An antibiotic, 1% penicillin-streptomycin, was used to prevent bacterial contamination of cell cultures. With the products mentioned above, the osteoblastic cells were cultured and frozen to be used for the three trials of the study.

To ensure cell attachment and to prepare the sample to receive the osteoblasts, the samples were submerged in 10% FBS supplemented medium overnight before the cells were seeded (Fig. 2a). After 24 hours, the zirconia and titanium samples were transferred to a 12-well plate. 1 ml MEMα medium was added to the titanium samples and control (glass slide) and 2.0 ml of the medium was added to the zirconia samples to fully cover the materials. The previously cultured MC3T3-E1 was detached by Trypsin/EDTA and the cell population was counted and adjusted to 15 × 10⁴/ml. Next, 1.0 ml of cell suspension was added to each well and incubated for 24 h (Fig. 2b). The materials were then observed for attachment by using a confocal microscope the following day and using LIVE/DEAD ® Viability/Cytotoxicity Assay Kit *for mammalian cells* (Life technologies Limited, Molecular Probes, USA) according to manufacturer’s instruction. The working solution was prepared in phosphate-buffered saline and pipetted into the wells containing osteoblasts on titanium, glass slide and zirconia surfaces. The samples were incubated for 30–45 minutes at room temperature. Using fine-tipped forceps, the incubated zirconia and titanium samples were inverted and mounted on the wet coverslip with 10 µL of D-PBS on the microscope slide. The labeled samples were viewed under a fluorescence microscope (Olympus FluoView™ FV1000 Confocal Microscope, PA, USA) using various magnifications.

Following the confocal microscopy, the samples were drained, washed twice with PBS and fixed with 2.5% glutaraldehyde at 4°C for 4 h. The samples were then dehydrated
through a series of graded alcohol solutions. The samples were allowed to dry at room
temperature before the SEM analysis.

**Statistical analysis**

Descriptive statistics was used to calculate the mean values, the ranges and the standard
deviations of the data obtained from the shear bond strength tests. The mean RAS of each test
group was analyzed using a two-way ANOVA with the RAS as the dependent valuable and
surface treatments and storage conditions as the independent valuables (p = 0.05).

**Results**

Boron carbide had produced the highest surface roughness compared to alumina and
silica-coated alumina powders exhibiting the lowest values (Table 1). The characteristics of
the surface morphology viewed under SEM, such as undercuts and crevices were much more
pronounced on the surface modified titanium surface compared to the zirconia surfaces. The
surface atomic concentration of the observed elements (Si, Al, B, O, C, Ni, Mg, Ag, Al)
varied insignificantly among the two materials on EDX examination. B and N were detected
if the concentration was not too low and beyond the detection limit of our EDX analyzer.

There was a significant difference (p=0.026<0.05) when the mean RAS values were
compared statistically among the storage conditions and different sand blasting materials
within zirconia (Fig. 1) and titanium (Fig. 2). While comparing the mean RAS of titanium,
specimens grit-blasted with silica-coated alumina powder showed the highest SBS in dry
storage and as thermo-cycled conditions (dry: 15.05 ±3.1 MPa, thermocycled:15.24± 4.21
MPa) whereas alumina powder exhibited higher SBS in wet conditions (16.10 ± 4.12 MPa).
Whereas in zirconia, specimens grit-blasted with silica-coated alumina powder produced the
highest SBS in all three storage conditions (dry: 10.32 ± 2.08 MPa; wet: 15.14 ± 2.66 MPa, thermo-cycled: 9.80 ± 2.80 MPa). In both of the substrate materials, boron carbide produced the lowest shear bond strength with the resin. The samples aged by thermo-cycling exhibited the lowest SBS values. Cohesive failure at resin was the least among the group (Table.2). Spontaneous debonding was observed at boron carbide specimens after thermocycling for zirconia, and after dry storage for titanium.

The cell viability was good in almost all of the surface modified samples viewed under a confocal microscope (Fig. 3). A consistently higher number of cells were attached in the samples grit-blasted with alumina powder among all the three trials performed. However, samples grit-blasted with silica-coated alumina powder, boron carbide powder and silicon carbide powder showed comparably similar results. The difference in morphology between the cells attached on the control and on the samples observed under SEM (Fig. 4) owes to the difference in the surface topography between the control and samples. In particular, cells exhibited a typical osteoblast type, which appears cuboidal with many lamellipodia and filopodia extensions. However, fewer cytoplasmic extensions and filopodia on zirconia surface were evident when compared with the titanium surface.
Discussion

The general results for both titanium and zirconia samples suggested that the mean RAS was highest for the surfaces grit-blasted with silica-coated alumina powder and alumina powder. Many studies have already confirmed the efficiency of using silica-coated alumina particles as a grit-blasting material to improve the surface’s bond strength to resin cements (Özcan & Vallittu 2003; Piwowarczyk et al. 2004). The current study reiterates the results observed from previous studies and, in addition that high Mohs hardness boron carbide abrasive material gave higher $R_a$ but was the least effective in eliciting sufficient RAS among the four tested powders. Therefore, it could say that the type of grit-blasting material used, but not the roughness, does influence the adhesive strength.

Among the material groups, resin-titanium bonding was stronger than resin-zirconia bonding, in general. This may be attributed more to the physical properties and surface chemistry of somewhat softer titanium and zirconia individually rather than the grit-blasting materials. All the samples grit-blasted with different powder materials almost unanimously exhibited a predominantly adhesive failure. This said, the grit-blasting materials might not have affected the mode of failure. Further studies using some other adhesive systems, such as novel silane primers, could be used to draw a clearer picture regarding the effects of the type of grit-blasting material on the mode of failure. Such silane primers might show different behavior on surfaces grit-blasted with the four grits studied above (Matinlinna & Lassila 2010; Matinlinna et al. 2007).

It was observed that sufficient osteoblastic adhesion was evident on all three trials experienced. The substrate materials grit-blasted with alumina powder exhibited a rich viable cell attachment compared to the other surfaces. However, some trials exhibited
inconsistencies. The fluorescent staining solvent might have expelled the insecurely attached cells from the surfaces prior to the observation. In conclusion, the surfaces modified by grit-blasting with alumina yielded a satisfactory layer of viable osteoblastic cell attachment despite the other surfaces showed comparably high attachment. The inconsistency of the results in the three conducted trials demands more subsequent experiments for a more definite estimation, and quantitative tests on osteoblast using agents such as MTT, alizarin red staining and ALP should be included in the near future studies. A further clarification about surface chemistry and cell adhesion is necessary.

Grit-blasting was the only surface modification that was performed and some other surface treatments could be performed to give data to make a definite conclusion. Nevertheless, grit-blasting has produced roughened surfaces and the chemical composition on the surface consists majorly of zirconium or titanium with trace amounts of elements (e.g., silicon) deposited. These elemental compositions may not have been enough to analyze the effect of the type of sand blasting material on the initial osteoblastic cell attachment. It is possible that the above results may have been incurred based only on the surface topographical modifications. Indeed, from the SEM, the osteoblasts spread to grow without any preferential direction in all grit-blasted surfaces. Grit-blasting in conjunction with other surface chemical modifications might yield a more definite result and should be considered as future research. Grit-blasting with all the four powders deemed to give possibly effective sites for osteoblastic cell attachment without denaturing the cell which was confirmed by confocal microscopy and SEM analysis.

In this laboratory study, we aim to compare four grits as a pretreatment on dental implant materials for resin and osteoblastic adhesions. From the data obtained, we might say
that silica-coated alumina powder or alumina powder when applied to abrade the surfaces of titanium and zirconia produced the highest adhesive strength with resin cement. In addition, the most abundant osteoblastic cells attachment was observed on the surfaces abraded with alumina powder. In overall, and as a suggestion, a one-piece dental implant made of titanium or zirconia could be grit-blasted with silica-coated alumina powder or alumina powder on the abutment surface and with alumina powder on the fixture screw surface to provide the highest resin bond strength and osteoblastic attachment, respectively.

This all said the hypothesis was partially rejected as there was a significant difference among the specimen group in terms of their RAS values. Although, the initial osteoblastic cell attachment experiment demands further trials to draw a definitive conclusion.

**Conclusions**

Surface treatment of zirconia and titanium by grit-blasting with four different sand powders changed the surface topography and composition. The resin adhesive strength varied significantly with samples grit-blasted with various grits. Highest adhesive strengths of both substrates were obtained for grit-blasting with silica-coated alumina or alumina powders. Osteoblastic adhesion on the grit-blasted surfaces varied with four different sand powders, that maximum osteoblastic attachment was observed for sample surfaces grit-blasted with alumina powders.

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References


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Figure 1. Box plot showing the adhesive strength values for resin-Zirconia bonding. Red and black lines in the grey 75% percentile boxes denote the mean and median SBS, respectively. × denotes the original data. Three specimens were spontaneously debonded for boron carbide during thermocycling. Abbreviation: SiC: Silica Carbide, BC: Boron Carbide, Dry: dry storage in a dry desiccator for 24 hours, Wet: stored in deionized water at 37°C in an incubator for 30 days, TC: thermo-cycling for 6000 cycles with a 20 second immersion time between two deionized water baths of temperatures 5.0 ± 0.5°C and 55.0 ± 0.5°C according to ISO 10477.
Figure 2. Box plot showing the adhesive strength values for resin-Titanium bonding. Red and black lines in the grey 75% percentile boxes denote the mean and median RAS, respectively. × denotes the original data. One specimen was spontaneously debonded for boron carbide during dry storage. Abbreviation: See Figure 1
Figure. 3. Confocal images of the osteoblastic cells attached on (a) zirconia and (b) titanium grit-blasted with silica-coated alumina; (c) zirconia and (d) titanium grit-blasted with alumina; (e) zirconia and (f) titanium grit-blasted with silicon carbide powder; (g) zirconia and (h) titanium grit-blasted with boron carbide powder.
Figure. 4. SEM images of the attached osteoblastic cells on (a) zirconia and (b) titanium surfaces grit-blasted with silica-coated alumina; (c) zirconia and (d) titanium surfaces grit-blasted with alumina powder; (e) zirconia and (f) titanium surfaces grit-blasted with silicon carbide powder; (g) zirconia and (h) titanium surfaces grit-blasted with boron carbide powder.
### Tables

Table 1. The mean $R_a \pm SD$ (μm) of the surface modified (a) Zirconia and (b) Titanium surfaces with various grits

<table>
<thead>
<tr>
<th>Grit-blasting materials</th>
<th>Silica-coated alumina</th>
<th>Alumina</th>
<th>Silicon carbide</th>
<th>Boron carbide</th>
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<td>Zirconia</td>
<td>0.41 ± 0.03</td>
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<td>Titanium</td>
<td>0.69 ± 0.01</td>
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Table 2. The modes of failure in resin adhesion of (a) Zirconia and (b) Titanium

(a) Zirconia

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<thead>
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(b) Titanium

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