Short Communication

Biomechanical and histologic evaluation of non-washed resorbable blasting media and alumina-blasted/acid-etched surfaces

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Abstract

Objectives: To compare the biomechanical fixation and histomorphometric parameters between two implant surfaces: non-washed resorbable blasting media (NWRBM) and alumina-blasted/acid-etched (AB/AE), in a dog model.

Material and methods: The surface topography was assessed by scanning electron microscopy, optical interferometry and chemistry by X-ray photoelectron spectroscopy (XPS). Six beagle dogs of $\sim$1.5 years of age were utilized and each animal received one implant of each surface per limb (distal radii sites). After a healing period of 3 weeks, the animals were euthanized and half of the implants were biomechanically tested (removal torque) and the other half was referred to nondecalcified histology processing. Histomorphometric analysis considered bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO). Following data normality check with the Kolmogorov–Smirnov test, statistical analysis was performed by paired *t*-tests at 95% level of significance.

Results: Surface roughness parameters S_a (average surface roughness) and S_q (mean root square of the surface) were significantly lower for the NWRBM compared with AB/AE. The XPS spectra revealed the presence of Ca and P in the NWRBM. While no significant differences were observed for both BIC and BAFO parameters ($P>0.35$ and $P>0.11$, respectively), a significantly higher level of torque was observed for the NWRBM group ($P=0.01$). Bone morphology was similar between groups, which presented newly formed woven bone in proximity with the implant surfaces.

Conclusion: A significant increase in early biomechanical fixation was observed for implants presenting the NWRBM surface.

Early in the 1980s, implant surface was identified as one of the six important factors for successful osseointegration (Albrektsson et al. 1981). Since then, efforts to engineer surface topography and chemistry that ultimately improve bone healing and reduce waiting times between device placement and functional loading have gained momentum and are currently regarded as a topic of high interest in implant dentistry (Albrektsson & Wennerberg 2004; Coelho et al. 2009).

To date, the existing variety of manufacturing processing techniques is so extensive that attempts to classify surfaces by modification method has become a difficult task (Dohan Ehrenfest et al. 2010). Therefore, a comprehensive codification system has been developed where surface characterization, made with standard analytical tools, describes the chemical composition (i.e. the composition of the bulk material and its chemical or biochemical modifications) and physical characteristics of the surface (topography at the micro- and nanometer scales) (Dohan Ehrenfest et al. 2010). However, it is known that surface topography changes with the varied processing techniques, may alter the surface chemistry and physics, although inadvertently (Wennerberg & Albrektsson 2009).

Among surface modifications altering both chemistry and topography, coating with hydroxyapatite or other CaP compositions has been the focus of several investigations (Wennerberg & Albrektsson 2009). Part of this interest is due to the fact that these elements are the same basic components of natural bone and coatings can be applied onto the implant surfaces by various industrial processing methods (Coelho et al. 2009). Chemistry modifications have been emphasized in the past with PSHA coatings, but long-term evaluations showed a compromise in the bond between the PSHA and the titanium surface leading to debonding in addition to non-

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uniform dissolution/degradation (Kay 1992; Ong et al. 2004; Yang et al. 2005). Subsequent surface bioceramic coatings that result in substantially thinner coating thicknesses include pulsed laser deposition (Kim et al. 2005), ion beam-assisted deposition (Granato et al. 2009), electrophoretic deposition (Lacefield 1998) and others. Alternative to continuous thin coatings, discrete crystalline depositions and the use of resorbable-blasting media (RBM) have also been used for the incorporation of Ca and P on and into the implant surfaces (Coelho et al. 2009).

The use of additional treatment to RBM surfaces with and without (non-washed RBMs) subsequent acid-etching has been investigated (Marin et al. 2010). Despite the differences in CaP amounts resulting from post-RBM blasting procedures, removal torque, bone-to-implant contact (BIC) and bone area fraction occupied (BAFO) were not significantly different at early implantation times *in vivo* (Marin et al. 2010). Because measured roughness parameters (S_a – average surface roughness and S_q – mean root square of the surface) were not significantly different between these surfaces (RBM and RBM + acid-etching), it can be suggested that the amount of CaP on the RBM surface was neither beneficial nor detrimental to the studied surfaces. Thus, this study hypothesized that an increased amount of CaP in a non-washed RBM surface (NWRBM) would result in an increased removal torque, BIC and BAFO relative to an alumina-blasted/acid-etched surface (AB/AE).

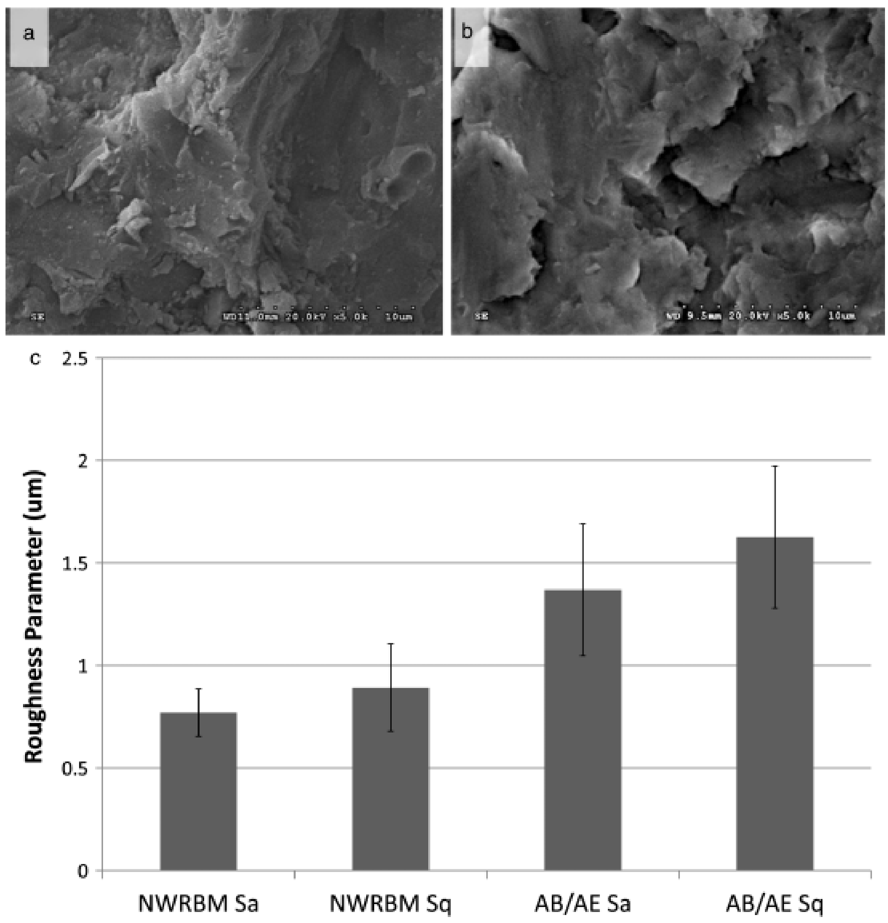
Materials and methods

This study utilized 3.75 mm in diameter by 10 mm length NWRBM and AB/AE-treated implant surfaces (Touareg, Adin Dental Implants Systems Ltd., Afula, Israel). Six implants of each surface were referred to physicochemical characterization. The surface topography was assessed by scanning electron microscopy (SEM) (Philips XL 30, Eindhoven, the Netherlands) at × 5000 magnification and an acceleration voltage of 20 kV ($n = 3$ per surface). Roughness parameters S_a and S_q were evaluated by optical interferometry (IFM) (Phase View 2.5, Palaiseau, France). Three implants of each surface were evaluated at the flat region of the implant cutting edges (three measurements per implant) and to separate roughness from waviness and shape for digital 3D measurements, a high-pass Gaussian filter of 50 μm × 50 μm was utilized (Leach 2009). Statistical analysis at 95% level of significance was performed by one-way ANOVA.

Chemical assessment was performed by X-ray photoelectron spectroscopy (XPS). The implants were inserted in a vacuum transfer chamber and degassed to 10^{-7} torr. The samples were then transferred under vacuum to the XPS spectrometer (Kratos Axis 165 multi-technique, Kratos Analytical Inc., Chestnut Ridge, NY, USA). Survey spectra were obtained using a 165 mm mean radius concentric hemispherical analyzer operated at a constant pass energy of 160 eV for survey and 80 eV for high-resolution scans. The take-off angle was 90° and a spot size of 150 μm × 150 μm was used. The implant surfaces were evaluated at various locations (three per implant).

For the animal model, eight beagle dogs of ~ 1.5 years of age were utilized. Following the approval of the ethics committee of the Universidade Federal de Santa Catarina, each animal received one implant of each surface per limb (radii sites) ($n = 16$ implants in total for the experiment). Before general anesthesia, IM atropine sulfate (0.044 mg/kg) and xilazyne chlorate (8 mg/kg) were administered. A 15 mg/kg ketamine chlorate dose was then utilized to achieve general anesthesia.

Surgical procedures for bone access and wound closure have been described in detail elsewhere (Coelho et al. 2010; Marin et al. 2010). For the *in vivo* model, 16 implants of each surface were utilized. In every radius, the starting implant surface was interchanged to minimize bias from different implantation sites, which allowed the comparison of the torque results of the same number of implant surfaces at 3 weeks per limb, surgical site (1 or 2) and animal. Implant placement followed the manufacturers directions. Post-operative anti-biotic and anti-inflam-



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Chemical Element (%)	NWRBM	AB/AE
Al2p	1.04	3.94
C1s	32.91	40.25
Ca2p	9.84	0.4
O1s	41.27	37.79
P2p	8.28	0.24
Ti2p	3.01	9.22
V2p3	0.16	0.41

Fig. 1. Scanning electron micrographs of the (a) NWRBM surface depicting evidence of blasting particles, and (b) AB/AE. (c) Measurable roughness parameters (Sa and Sq) showed overall higher roughness profile for the AB/AE group compared with the NWRBM ($P < 0.001$). (d) Average chemical composition for the different surfaces as observed in the XPS spectra.

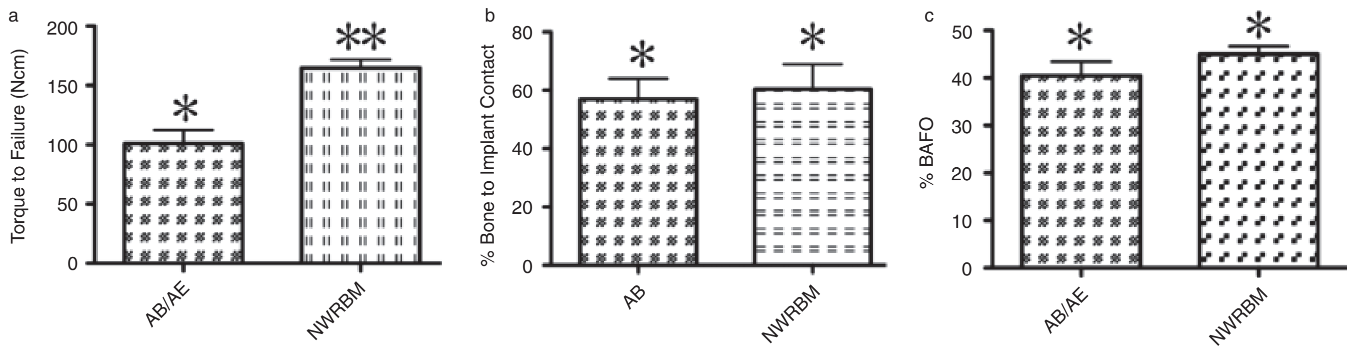

Fig. 2. (a) Significantly higher removal torque ($P = 0.01$) was observed for the NWRBM surface compared with the AB/AE, whereas (b) BIC and (c) BAFO ($P > 0.35$ and $P > 0.11$, respectively) statistics summary (mean ± 95% confidence intervals) presented no significant differences.

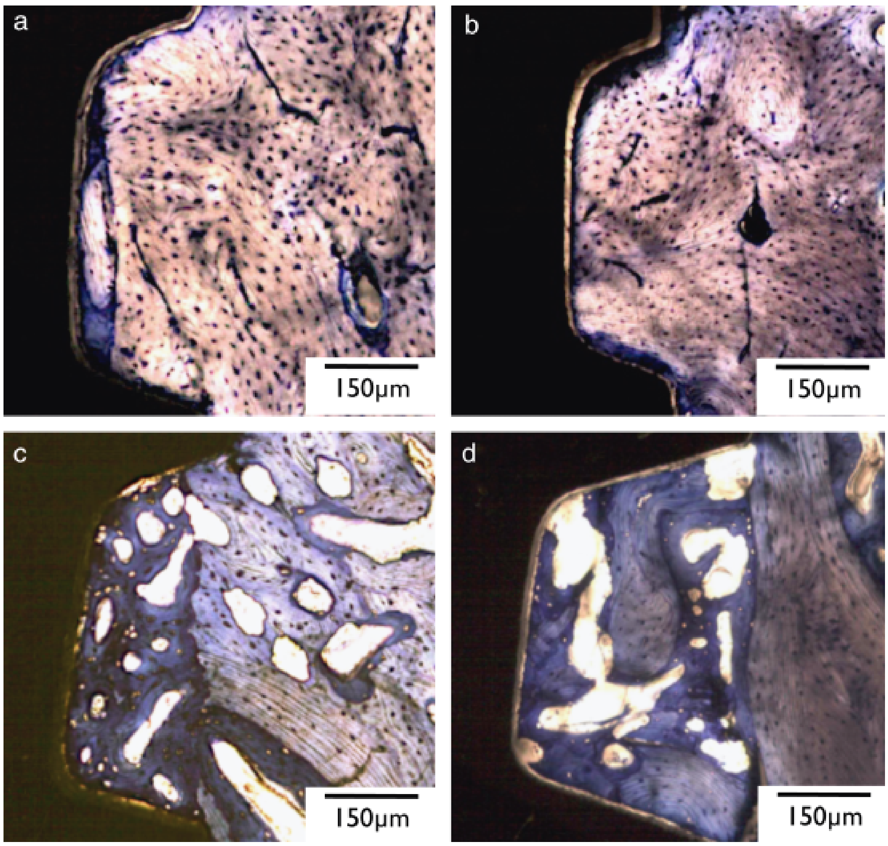

Fig. 3. Optical microscopy revealed similar bone to implant response for the NWRBM (a – cortical and c – trabecular) and AB/AE (b – cortical and d – trabecular) where newly formed woven bone is observed in proximity with both surfaces.

matory medication included a single dose of benzyl penicillin benzatine (20 UI/kg) IM and ketoprofen 1% (1 ml/5 kg). The animals were euthanized after a post-surgical period of 3 weeks by anesthesia overdose and the limbs were retrieved by sharp dissection. The soft tissue was removed by surgical blades, and initial clinical evaluation was performed to determine implant stability. If an implant was clinically unstable, it was excluded from the study. Half of the implants were biomechanically tested (removal torque) and the other half referred to non-decalcified histology processing as reported previously (Coelho et al. 2010; Marin et al. 2010). Histomorphometric analysis considered BIC and BAFO. Following data normality check by the Kolmogorov–Smirnov test (where all groups presented $P > 0.1$), statistical analysis was performed by paired *t*-tests at 95% level of significance.

Results

Both implant surfaces' electron micrographs and their resulting IFM surface roughness parameters are presented in Fig. 1. The surface texture observed in the SEM micrographs at high magnification showed evidence of residual blasting media particles on the NWRBM (Fig. 1a), but not of embedded alumina particles on the AB/AE (Fig. 1b). S_a and S_q values were significantly lower for the NWRBM relative to AB/AE (Fig. 1c) ($P < 0.001$). The XPS spectra revealed the presence of Ca and P in the NWRBM (80% TCP 20% HA) (Fig. 1d).

No complications during animal surgical procedures and follow-up were observed and all implants were clinically stable immediately after euthanization. While no significant differences were observed for both BIC and BAFO parameters ($P > 0.35$ and $P > 0.11$, respectively), a significantly higher level of torque was observed for the NWRBM group ($P = 0.01$) (Fig. 2). Bone morphology was similar between groups, which presented newly formed woven bone in proximity with the implant surfaces (Fig. 3).

Discussion

Surface roughness assessment by IFM revealed higher S_a and S_q for the AB/AE compared with the NWRBM, which is in agreement with a previous investigation (Marin et al. 2010). Although higher bone interlocking and removal torque would be expected for the rougher AB/AE surface, no significant differences were observed, in that previous study, when compared with the RBM and RBM + acid-etched surfaces (Marin et al. 2010). These results suggest that either CaP amounts were too low and/or that roughness of surfaces were equally effective in providing resistance to removal torque. To further evaluate this question, a sequel investigation assessed the resulting BIC and removal torque of an RBM surface now with significantly higher S_a and S_q compared with an AB/AE, and remarkably, no differences were observed in the canine model at either 2 or 4 weeks *in vivo* (Bonfante et al. in press).

Considering the questions raised in previous RBM studies, the rationale of the present study was to further evaluate the effect of increased CaP amounts by a non-washing procedure of an RBM surface when compared with a AB/AE surface. Despite the higher roughness of the latter surface, which fell in the moderately rough range shown previously to present the strongest bone response (S_a approximately 1.5 μm) (Wennerberg et al. 1995a, 1995b, 1996; Wennerberg & Albrektsson 2009), the resulting higher removal torque for the smoother NWRBM surface suggests that the residual CaP was beneficial to implant biomechanical fixation, but not for BIC and BAFO. As emphasized previously in the literature, interpretation of static histomorphometric parameters such as BIC and BAFO should be made with caution because they are indicators of osseointegration that do not accurately reflect bone/implant biomechanical interaction (Coelho et al. 2009).

The histomorphologic sections depicted bone in close contact with both implant surfaces in trabecular and cortical bones at 3 weeks, suggesting that surfaces were biocompatible and osseoconductive.

Higher removal torque was observed for the NWRBM surface compared with AB/AE, but not significantly different BIC and BAFO measurements, leading to partial acceptance of the present study hypothesis. Because RBM processing of surfaces with post-blasting treatments that resulted in varied amounts of Ca and P on the surface (smaller than in the present study) have been attempted previously, the results obtained in the present study are encouraging and further investigation concerning NWRBM's composition and associated *in vivo* performance is desirable.

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