Acid and Alkaline Etching of Sandblasted Zirconia Implants: A Histomorphometric Study in Miniature Pigs

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ABSTRACT

Background: Zirconia (ZrO_2) has received interest as a dental material; however, little information is available on the impact of surface modifications on the osseointegration of zirconia implants.

Purpose: The aim of the present study was to determine the effect of acid or alkaline etching of sandblasted ZrO_2 implants on bone apposition in vivo.

Methods: Cylindrical ZrO_2 implants with two circumferential grooves were placed in the maxilla of 12 miniature pigs. Biopsies were harvested after 1, 2, 4, and 8 weeks of healing. Undecalcified toluidine blue-stained ground sections were produced. The bone-to-implant contact, the bone area, and the presence of multinucleated giant cells were determined by histomorphometry. An uncorrected explorative statistical analysis was performed.

Results: Acid etching but not alkaline etching of sandblasted ZrO_2 implants caused more bone-to-implant contact than sandblasted ZrO_2 implants. The bone area was unaffected by the surface modifications. Acid and alkaline etching both increased the formation of multinucleated giant cells at the implant surface.

Conclusions: This study provides a scientific basis to further investigate the impact of acid etching of sandblasted ZrO_2 implants on osseointegration and the role of multinucleated giant cells in this process.

KEY WORDS: dental implant, histology, histomorphometry, macrophages, multinucleated giant cells, osseointegration, zirconia

INTRODUCTION

Implants of commercially pure titanium (cpTi) with moderately rough surfaces are widely used in dentistry.[1] Dental implants made of titanium yield high survival rates.[2–5] However, the grayish color can become visible in the aesthetic zone of patients with a thin gingival biotype.[6,7] Bone resorption with soft tissue recession can even worsen this situation. Rare allergic reactions to titanium have further raised the demand for alternative materials.[8] Thus, there is a need for tooth color-like implants with favorable biocompatibility.

Zirconia (ZrO_2) has been recently introduced to implant dentistry.[9–11] ZrO_2 has a high bending strength and fracture toughness, resistance to corrosion and wear,[12] biocompatibility,[13] and minimal ion release.[14] The biomechanical stability of ZrO_2 is increased by the addition of tetragonal polycrystals of yttrium.[15] Ball heads of hip prostheses can be made from ZrO_2.[16] In implant dentistry, the ivory color renders ZrO_2 useful

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for aesthetic restorations.[17] However, the impact of ZrO_2 and its surface morphology on the complex process of osseointegration is only beginning to be understood.[18]

Preclinical studies have revealed bone apposition on ZrO_2 with various surface modifications, including sandblasting,[19,20] etching,[19,21,22] sintering, and coating.[23,24] Bone apposition can be surprisingly similar for different modifications of the ZrO_2 surface.[19,20,23–25] Subtle changes of the surface can, however, have an impact on bone formation; therefore, further studies are required. Moreover, selection of preclinical models and the anatomy of the implant can affect the outcome, which is usually bone apposition. However, the presence of multinucleated giant cells should also be evaluated.

Multinucleated giant cells (MNGCs), for example, were abundant on Ti6Al4V alloy but only occasionally found on titanium–zirconium and cpTi implants in a minipig model.[26] Subcutaneous models were also established to understand the impact of biomaterials on the formation of MNGCs from macrophages.[27] The role of macrophages during bone regeneration is not yet clear. Macrophages have multiple functions during wound healing,[28] but MNGCs are also the hallmark of a foreign body reaction.[29] Because their role in osseointegration is still not understood, reporting on MNGCs is important.

Hence, an explorative study was performed to examine a possible impact of surface modifications – alkali and acid etching of sandblasted ZrO_2 implants – on bone apposition and the formation of MNGCs in the jaw of miniature pigs.

MATERIAL AND METHODS

Implant Design

Cylindrical ZrO_2 implants had a length of 6.0 mm, a core diameter of 2.7 mm, and three rings with an outer diameter of 4.2 mm (Figure 1). The two circular grooves had a depth of 0.75 mm and a height of 1.8 mm. All implants were identical in shape but differed in their surface characteristics. The surface of all implants was sandblasted with Al_2O_3 with a particle size of 255 to 500 µm under a pressure of 3.5 bar. Implants were divided into the following three surface treatment groups: (i) acid-etched sandblasted implants, following an undisclosed protocol (SB-AC), (ii) alkali-etched and sandblasted implants, obtained with a hot solution of sodium hydroxide and potassium hydroxide for 24 hours (SB-AL); and (iii) sandblasted implants (SB). All implants were cleaned by ultrasound and heat-sterilized at a temperature of 134°C for 18 minutes. Morphological differences between the three surface topographies are shown in Figure 2.

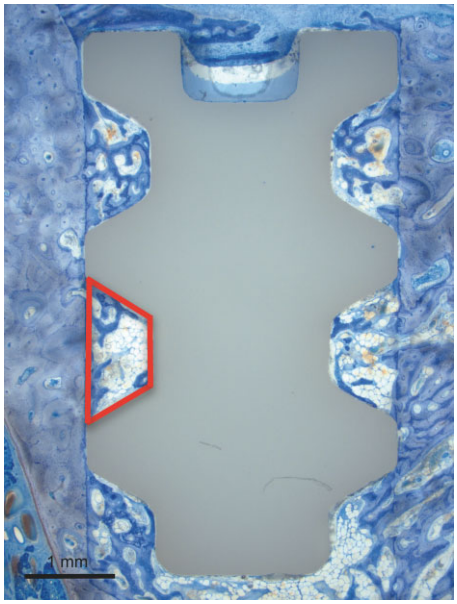

Figure 1 Longitudinal ground section showing the implant, peri-implant tissues, and the four implant grooves with marked region of interest.

Study Design and Surgery

A total of 12 adult (2- to 4-year-old) miniature pigs were used in this study. The husbandry and care of animals before, during, and after surgery was handled at the Surgical Research Unit ESI and at the Clinic for Large Animals, University of Bern, Switzerland. The animals received standard food and water ad libitum. The protocol of the study was approved by the Committee for Animal Research, State of Bern, Switzerland (Approval no. 82/10), using a study design that has been successfully utilized in previous studies.[26,30,31] Animals were premedicated using ketamine (intramuscular [i.m.] 20 mg/kg), xylazine (i.m. 2 mg/kg), atropine (intravenous [i.v.] 0,05 mg/kg), and midazolam (i.v. 0,5 mg/kg) to achieve intubation. Inhalation anesthesia was performed with isoflurane (1.0–1.5%). Fentanyl patches (5–10 µg/kg) were used for the intraoperative analgesia, and the animals received antibiotic prophylaxis for 3 days (Duplocillin LA, 12,000 U.I./kg; MSD Animal Health GmbH, Luzern, Switzerland).

This prospective, randomized, controlled experimental study was performed in three surgical phases.

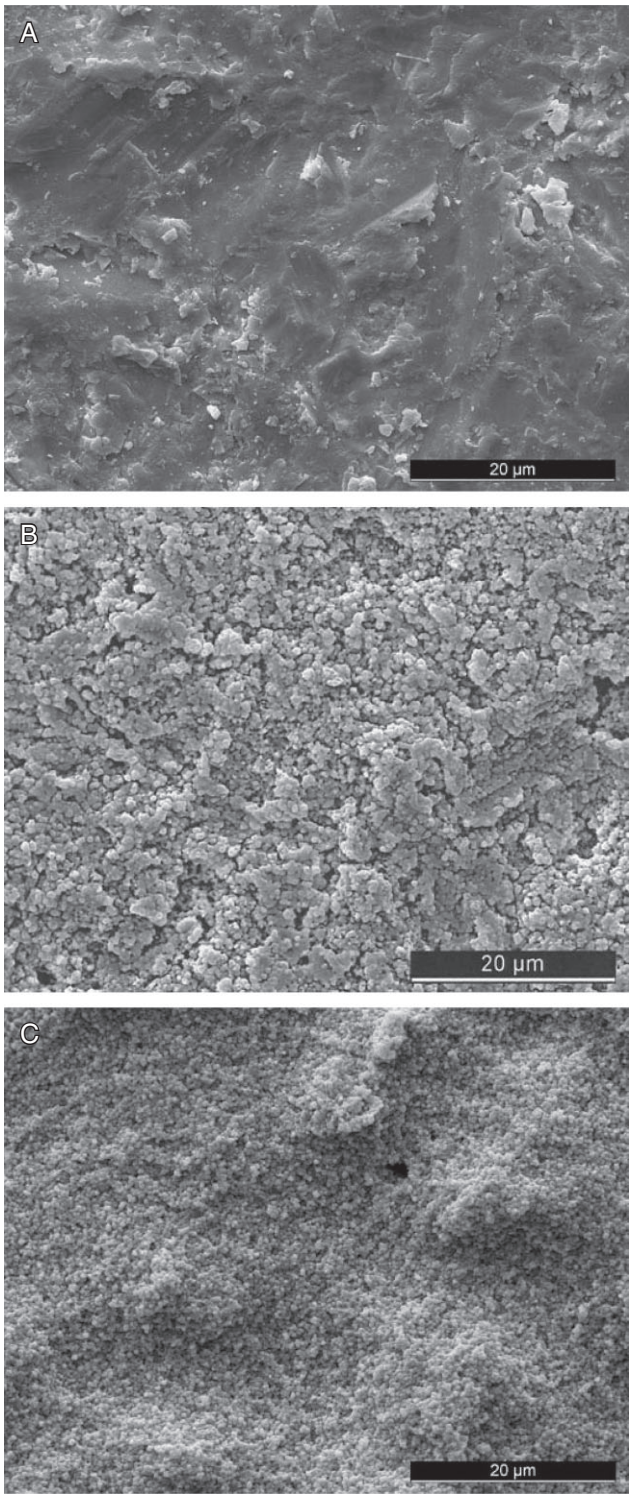

Figure 2 Scanning electron microscopic images of ZrO_2 implants showing the topographies of sandblasted (A), sandblasted and alkali etched (B), and sandblasted and acid etched (C) surfaces.

During the first surgical intervention, all six incisors in the maxilla were removed, and the sites were allowed to heal for at least 3 months. Implants were then placed during two surgical interventions on either side of the maxilla, according to a split-mouth design. Implants were placed 1 mm subcrestally with good primary stability, provided by the press fit of the implants with the bone walls of the prepared implant beds. Depending on the anatomical situation, three or four implants were inserted on one side of the maxilla using a systematic random protocol. In six animals, implants were first placed on the left side and one week later also on the right side of the maxilla. The animals were sacrificed 1 week later, yielding healing periods of 1 and 2 weeks. In the other six animals, implants were first placed on the left side and 4 weeks later on the right side of the maxilla. The animals were sacrificed after another 4 weeks, yielding healing periods of 4 and 8 weeks.

Animals were sacrificed by intravenous injection of 20 mmol KCI. Immediately after death, soft tissues were removed to expose the edentulous area of the maxilla. Two bone blocks were produced for each animal with the use of an oscillating diamond-coated band saw. The block specimens were fixed in 4% formaldehyde combined with $CaCl_2$ prior to histologic preparation. A sample size of six implants per time period (12 animals; $n = 72$ in total; $n = 18$ per time period; $n = 6$ for each implant surface) is sufficient for an explorative statistical analysis.

Histological Preparation and Analysis

The specimens were rinsed in water, dehydrated in ascending alcohol fractions, and embedded in methylmethacrylate.[32] The details of the histological processing have been described in previous studies.[33,34] Each implant was sectioned parallel to its longitudinal axis in the vestibulo-oral direction, resulting in three undecalcified sections of ~500 µm thickness. The sections were ground to a final thickness of 80 µm and superficially stained with toluidine blue. The two central-most sections were used for descriptive and morphometric analyses. The region of interest was defined by the surface of the implant groove and the extension of the outer implant diameter (Figure 1). The percentages of the implant surface covered by total bone (BIC) and by MNGCs (MIC) were determined at all time points. The bone area (BA) fractions within the implant grooves were assessed at 8 weeks. Histomorphometric analysis was performed directly in the light microscope by intersection counting, using an integrative eyepiece with parallel sampling lines at a magnification of ×250 using a square grid (distance between test points = 40 µm at a magnification of ×250).

Statistical Analysis

The Friedman test with a post hoc Wilcoxon test for pairwise comparisons was used to test for differences between surface characteristics at each healing time. No correction for *p* values was done. The correlation between MIC and BIC was performed with Spearman's rank and Pearson's correlation coefficient. A Brunner–Langer F1_LD_F1 model was used with healing time as a whole plot factor and surface characteristic as a subplot factor. A significance level of 0.05 was chosen.

RESULTS

All 12 animals survived the surgical procedures and the subsequent observation periods without complications. Clinical inspection did not reveal any dehiscences or signs of infection at the surgical sites. At the time of sacrifice, no implant was lost, and all implants were in a correct submerged position.

Histological Analysis

Bony ingrowth into the implant grooves was already evident after 1 week for all three implant surface modifications (Figure 3). Newly formed bone consisted mainly of osteoid. Initial signs of bone mineralization were noticed for two implants with an SB-AL and two implants with an SB-AC surface. Loose connective tissue and coagulum remnants were generally present. Osteoid was present on one implant with an SB surface and three implants with SB-AC surfaces. The ratio between mineralized bone and osteoid increased steadily over time. At 2 weeks, mineralized bone and osteoid were found within the grooves of all implants (Figure 3), and BIC was noticed for all implants except one with an SB-AL surface. Bone trabeculae first consisted entirely of woven bone and were later reinforced by parallel-fibered bone. Although most of the implants demonstrated the presence of adipocytes within the grooves at 4 weeks (Figure 4), maturation of bone marrow adjacent to SB-AC implants was more advanced than for SB and SB-AL implants (Figure 5). With one exception – an implant with an SB-AL surface at 4 weeks – mature bone marrow within the grooves was not seen before 8 weeks.

Sparse MNGCs were already present after 1 week of healing on all implants, independent of the surface modifications (Figure 3). Already at 2 weeks, MNGCs

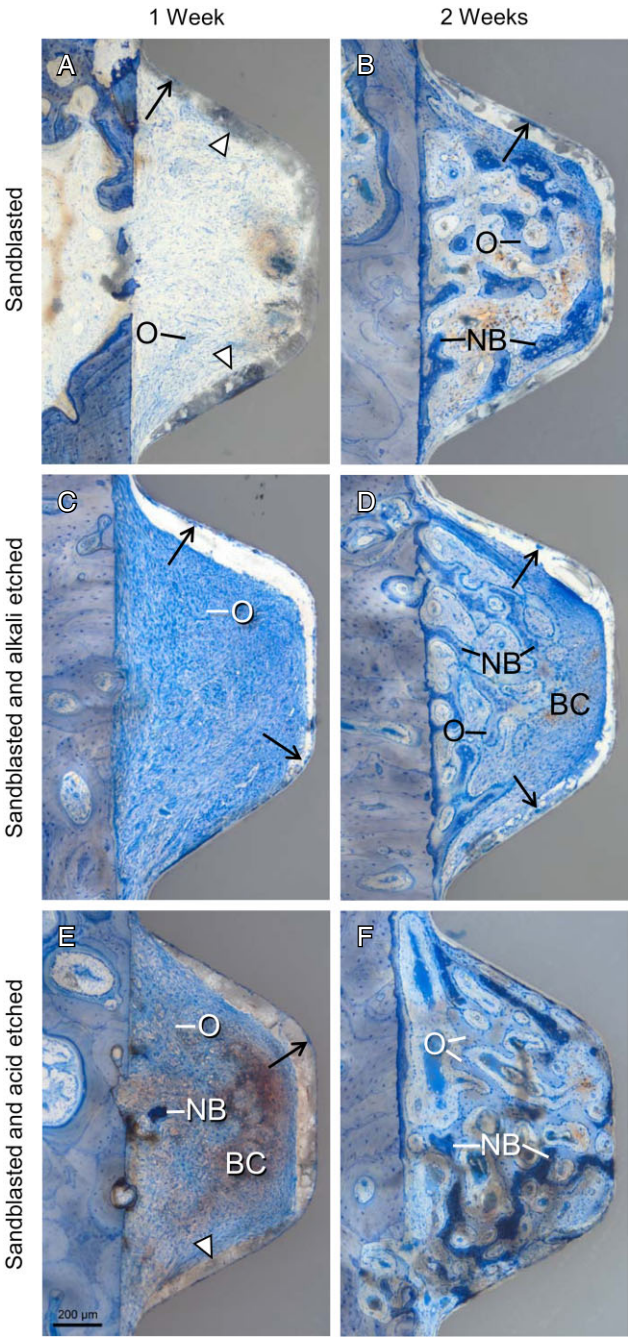

Figure 3 Histological sections illustrating the implant grooves delineated by the implant surface and the bony wall of pristine bone at 1 (A, C, E) and 2 (B, D, F) weeks for all three implant surfaces. After 1 week, most of the newly formed bone is osteoid (O) and the blood coagulum (BC) is present. After 2 weeks, the new mineralized bone (NB) is present close to the cut bone, and the newly formed bone matrix is mainly mineralized. Residual coagulum is still present. The bone-to-implant contact is very low for all three implant surfaces. The implant surfaces reveal tissue detachment from the implant surfaces with accumulation of liquor (arrowheads) and numerous multinucleated giant cells (arrows).

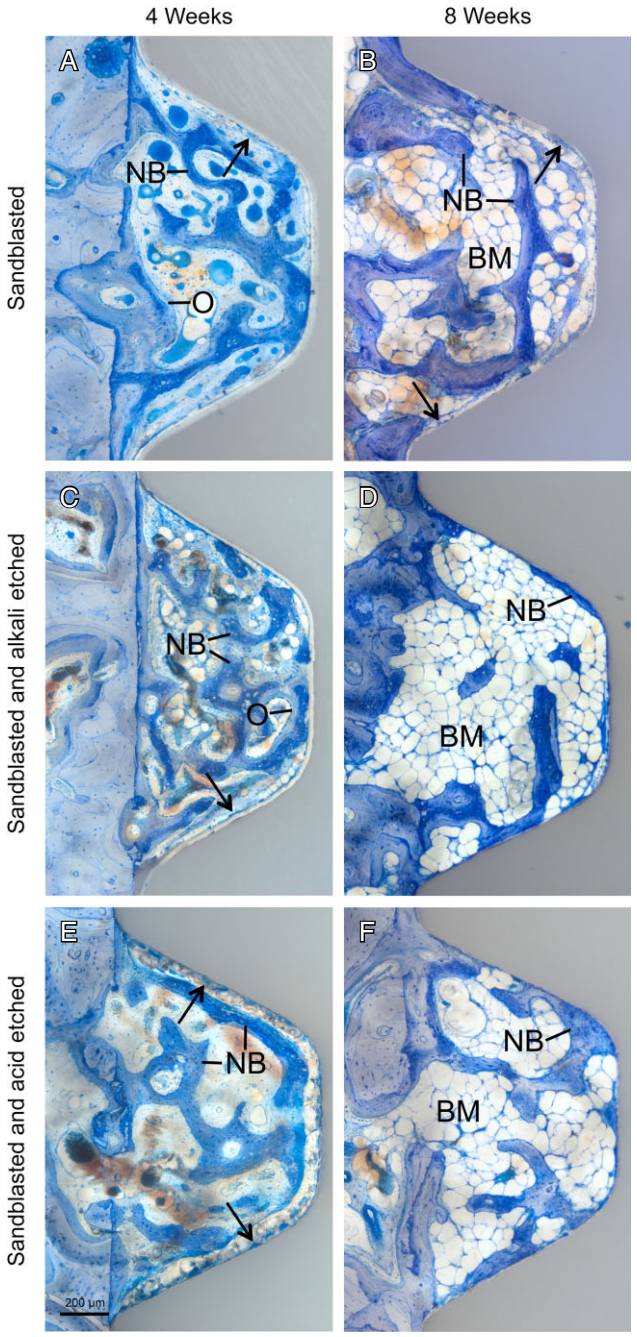

Figure 4 Histological appearance within the implant grooves at 4 (A, C, E) and 8 (B, D, F) weeks for all three implant surfaces. The grooves of all three implant surfaces are filled with approximately the same amount of new bone (NB). Most of the bone matrix is mineralized. After 8 weeks, bone is much more mature, as indicated by an increased ratio of mineralized bone to osteoid (O) and a higher maturity of bone marrow (BM). The bone-to-implant contact is higher for the implant surfaces that are both sandblasted and acid etched, whereas the sandblasted surfaces and the sandblasted and alkali etched surfaces demonstrate reduced bone-to-implant contact. All three implant surfaces reveal numerous multinucleated giant cells (arrows).

were large but flat, possessed two to three round nuclei, and lined a large portion of the implant surface (Figure 6). In some areas, MNGCs appeared to be in close contact with active osteoblasts. At 4 weeks, MNGCs demonstrated a different morphology, with an increased number of nuclei and a less flat appearance (Figure 6). This shape was predominantly observed

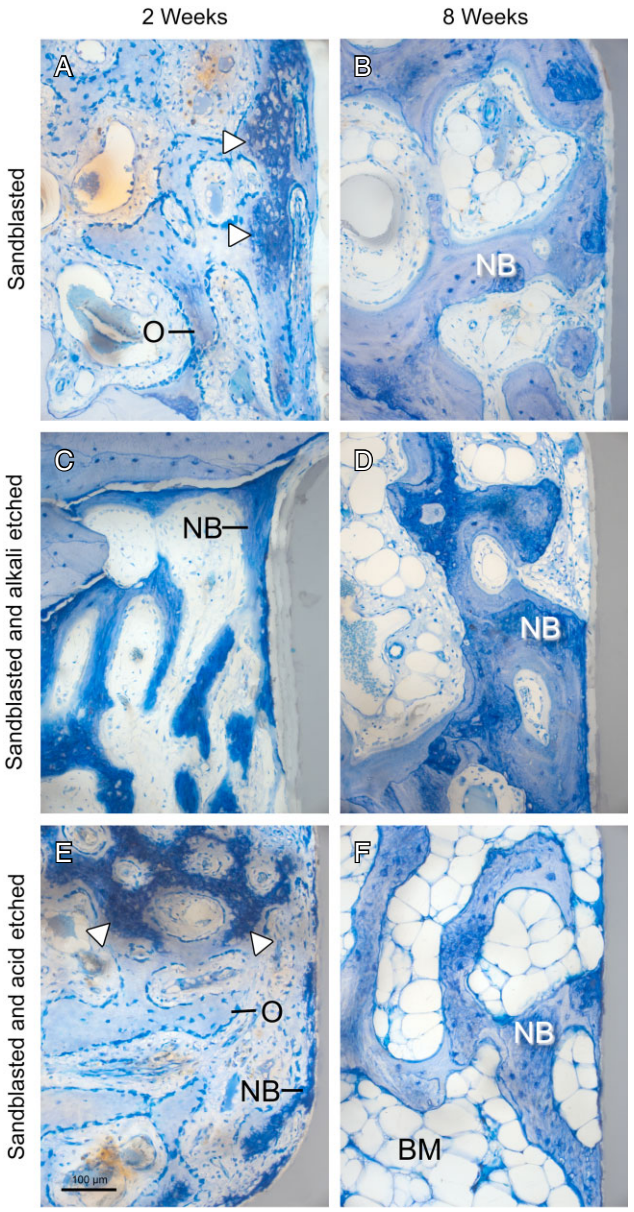

Figure 5 Detailed histological views within the grooves facing ZrO_2 implants. At 2 weeks (A, C, E), the osteoid (O) with initial mineralization (arrowheads) is deposited onto the implant surface. New mineralized bone (NB) is present close to the pristine bone. At 8 weeks (B, D, F), a layer of new mineralized bone covers the implant surface. Both the newly formed bone matrix and bone marrow (BM) are mature. Note the presence of fatty marrow tissue and fibrous marrow tissue.

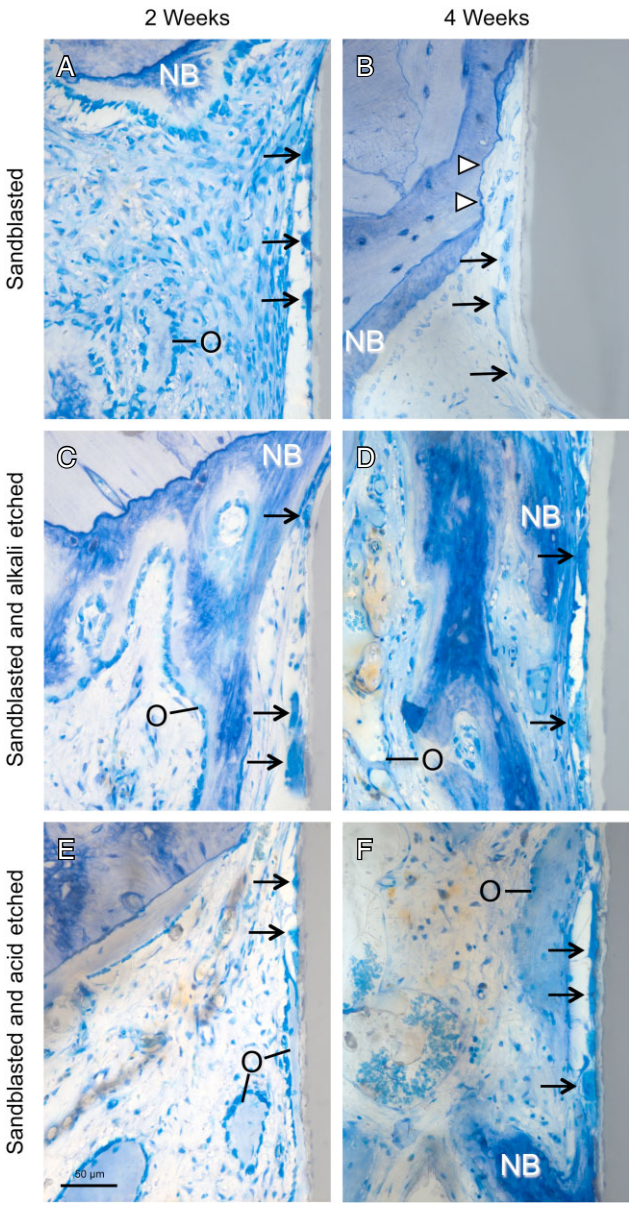

Figure 6 Detailed histological views within the grooves facing ZrO_2 implants at 2 and 4 weeks. Numerous MNGCs (arrows) line the large portion of ZrO_2 implants and the BIC is very low. At 2 weeks of healing, multinucleated giant cells (MNGCs) have a flat shape, with a large diameter distributed on the implant surface. MNGCs are in contact with the rim of osteoblasts secreting osteoid matrix (O). The loose connective tissue and the osteoid seem detached from the implant surface. At 4 weeks, MNGCs are rounder in shape, intervening between the osteoid or the new mineralized bone (NB) and the implant surface. The scalloped bone surface indicates resorptive activity (arrowheads).

when the cells were located close to newly formed bone. At 8 weeks, the morphology of MNGCs appeared to be similar as observed at 4 weeks, but their number tended to decrease. There were no inflammatory cells observed adjacent to MNGCs at any stage of healing.

Histomorphometric Analysis

The histomorphometric analysis showed that surface modifications had an impact on BIC (Table 1) with significant differences at week 2 ($p = .0062$) and week 8 ($p = .0155$). Post hoc testing with an uncorrected explorative statistical analysis revealed that acid etching but not alkaline etching of sandblasted ZrO_2 implants increased BIC compared with sandblasting alone (Table 2; Figure 7). The BA at 8 weeks for SB, SB-AL, and SB-AC surfaces was similar among the three groups (mean ±standard deviation, 45.18% ± 10.25, 43.95% ± 11.25, and 42.11% ± 15.57, respectively). Surface modifications also had an impact on MIC at a healing time of week 8 ($p = .0057$; Figure 7). Post hoc analysis suggested that both acid etching and alkaline etching of sandblasted ZrO_2 implants enhanced MIC compared with sandblasting alone (Table 2). Thus, acid etching of sandblasted ZrO_2 implants provoked the most BIC; however, both etching procedures also increased the formation of MNGCs at the implant surface.

Correlation analysis was performed to investigate the possible association of BIC and MIC. When considering all implants over all time points, the dependence between the variables was almost nil as indicated by Pearson's ($r = -0.01$) correlation coefficient. By giving ranks to the observations, the Spearman's ($r = 0.21$) correlation becomes weakly positive. When looking at healing time week 8, the scatter plot suggests that the three implant surfaces seem to behave differently (Figure 8). In particular for the acid etching of sandblasted ZrO_2 implants, the amount of MNGCs seems to be negatively associated with the BIC, but the data did not allow a powerful statistical analysis.

A nonparametric analysis of longitudinal data according to the Brunner–Langer model revealed that for BIC, healing time (week 1–week 8) and surface modification were significant ($p < .0001$) but not their interaction ($p = .39$; Table 3). The post hoc tests were significant for SB-AC ($p = .0012$), SB-AL ($p = .0022$), and SB ($p = .0012$) surfaces. For MIC, healing time and surface treatment were significant ($p < .01$; Table 3). The interaction term is also significant, that is, the different types of implant behave differently over time. Post hoc testing showed that acid ($p = .0047$) and alkaline ($p = .0022$) etching of sandblasted ZrO_2 – but not sandblasting alone ($p = .2343$) – became significant for MIC. Thus, the surface modification has an impact on the formation of MNGCs between week one and week eight.

TABLE 1 Percentage of Osteoid, Mineralized Bone, Total New Bone (Osteoid and Mineralized Bone), Soft Tissue and Multinucleated Giant Cells in Contact with the Surfaces of Three Implant Surfaces over Time. Data Are Shown as Means ± Standard Deviation

Time Point	Implant Surface	Osteoid	Mineralized Bone	Total New Bone	Soft Tissue	Multinucleated Giant Cells
Week 1	SB	0.28 ± 0.49	0.00 ± 0.00	0.28 ± 0.49	99.65 ± 0.44	6.55 ± 3.14
	SB-AL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	99.85 ± 0.18	4.29 ± 2.62
	SB-AC	1.16 ± 1.83	0.04 ± 0.05	1.2 ± 1.84	98.41 ± 1.99	11.91 ± 9.97
Week 2	SB	4.06 ± 3.25	2.72 ± 3.47	6.77 ± 5.81	93.23 ± 5.81	11.52 ± 10.81
	SB-AL	1.12 ± 3.96	2.01 ± 2.80	4.13 ± 6.18	95.87 ± 6.18	28.27 ± 20.51
	SB-AC	11.44 ± 6.32	12.97 ± 11.20	24.41 ± 17.06	75.59 ± 17.06	15.25 ± 13.6
Week 4	SB	4.08 ± 2.78	8.56 ± 3.90	12.64 ± 4.10	87.29 ± 3.96	22.40 ± 13.29
	SB-AL	2.66 ± 2.16	3.77 ± 2.71	6.43 ± 3.93	93.57 ± 3.93	48.65 ± 11.67
	SB-AC	3.45 ± 2.95	16.27 ± 15.24	19.73 ± 17.60	80.27 ± 17.60	44.46 ± 17.52
Week 8	SB	5.34 ± 2.94	24.73 ± 20.20	30.07 ± 19.32	69.93 ± 19.32	16.16 ± 7.58
	SB-AL	3.80 ± 1.47	17.93 ± 18.50	21.73 ± 18.46	78.27 ± 18.46	40.72 ± 14.46
	SB-AC	1.40 ± 1.06	44.42 ± 18.16	45.82 ± 17.71	54.18 ± 17.71	36.59 ± 13.02

SB = sandblasted; SB-AL = sandblasted and alkali etched; SB-AC = sandblasted and acid etched.

TABLE 2 Post Hoc Testing for Pairwise Comparison Per Surface Treatment (*p* Values)

Healing Period	Implant Surfaces	Total New Bone	Multinucleated Giant Cells
2 weeks	SB vs. SB-AL	0.4375	–
	SB vs. SB-AC	0.0313	–
	SB-AL vs. SB-AC	0.0313	–
8 weeks	SB vs. SB-AL	0.0625	0.0313
	SB vs. SB-AC	0.0313	0.0156
	SB-AL vs. SB-AC	0.0313	0.2188

SB = sandblasted; SB-AL = sandblasted and alkali etched; SB-AC = sandblasted and acid etched.

DISCUSSION

In the present study, the impact of surface modifications of sandblasted ZrO_2 implants on the process of osseointegration in the maxilla of miniature pigs was analyzed. The first main finding of our uncorrected explorative statistical analysis was that acid etching but not alkaline etching of sandblasted ZrO_2 implants caused more BIC than sandblasting of ZrO_2 implants alone. The second main finding was that acid and alkaline etching of sandblasted ZrO_2 enhanced the formation of MNGCs. Overall, these findings support the current knowledge that surface modifications of sandblasted ZrO_2 implants

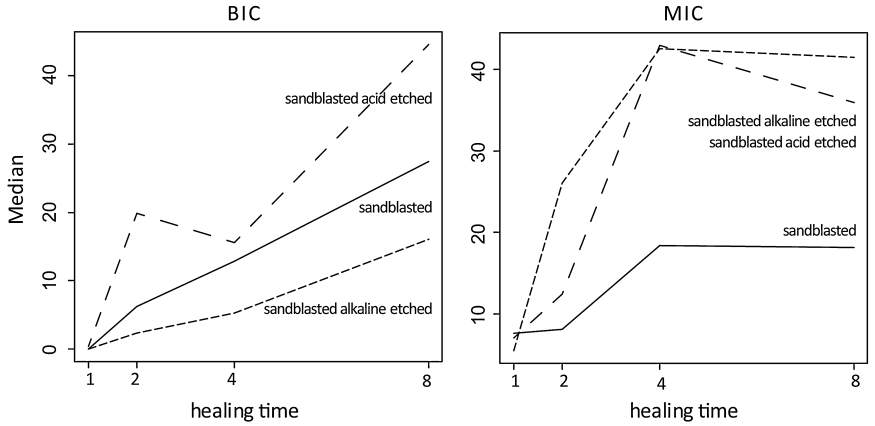

Figure 7 Histogram illustrating the effect of the implant surfaces on the percentage of bone-to-implant contact (BIC) and the percentage of implant surface along the grooves covered with MNGCs (MIC) over time.

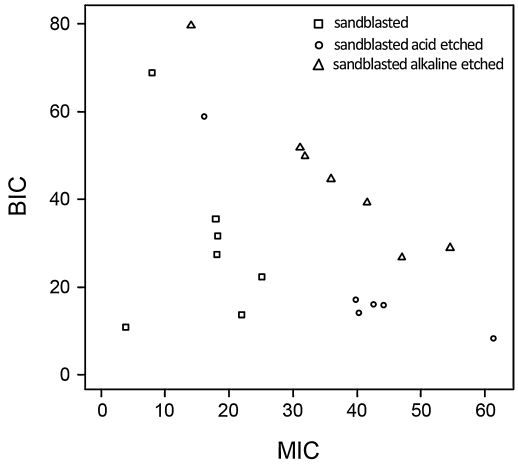

Figure 8 Scatter plot illustrating the correlation between the percentage of bone-to-implant contact (BIC) and the percentage of implant surface along the grooves covered with MNGCs (MIC) at the 8-week healing period.

can affect the process of osseointegration that possibly involves the formation of MNGCs. However, as the design of the present study only allowed an uncorrected explorative statistical analysis, the present findings should provide the scientific basis for the design of future studies.

Comparing the data to those of others, BIC were lower than those reported in other studies for sandblasted[19,20,35,36] or sandblasted and alkali-etched ZrO_2 implants.[19,37] However, differences in the percentage of BIC may be attributed to animal models,[20,37] site of implantation,[19,36] micro- and macro-design of the implants,[19,20,35–37] healing periods,[20,35] and surface modifications. Regarding the latter aspect, smaller blasting particle sizes can produce higher BIC values.[19,35] However, not all studies have specified the particle sizes.[20,36] Together, the findings suggest that parameters of osseointegration can be compared within a study but rarely between studies.

TABLE 3 Effect of Time, Implant Surface and Interaction Effect (Time and Treatment) on the Amount of Total New Bone and Multinucleated Giant Cells Deposited on the Implant Surfaces

	Total New Bone		Multinucleated Giant Cells	
Effect	Statistic	*p*	Statistic	*p*
Healing time	78.33	<.0001	19.94	<.0001
Surface treatment	11.13	<.0001	5.601	.0097
Interaction effect	0.936	.3893	7.728	.0020

Acid etching of ZrO_2 implants caused more BIC than sandblasting alone at 2 and 8 weeks of healing. This finding is however not valid for the 1-week and 4-week time points, suggesting that the supportive activity of the surface for bone formation is not robust. As this is an underpowered study with an uncorrected explorative statistical analysis, the data have to be interpreted with caution. Yet, the reason why the acid-etched sandblasted ZrO_2 surface performed better than the other two surfaces is not known. BA measures suggest that the overall process of bone formation is not impaired by the surface modifications tested. Future research can be undertaken to understand more about the effects of roughness and surface chemistry on the process of bone formation. Moreover, removal torque analysis should be considered in future studies to identify a possible correlation between biomechanical and histomorphometrical data on three ZrO_2 surface modalities.

The formation of MNGCs was expected based on the results of a recent preclinical study, with Ti6Al4V implants.[26] However, questions arise why acid and alkaline etching supported the formation of MNGCs on the implant surface. In vitro studies suggest that surface topography has an impact on macrophage activation and thus the secretion of proinflammatory cytokines and chemokines.[38] Although the surface roughness parameters in a recently published study were rather similar among the three examined surface modifications, the scanning electron microscope pictures clearly showed a different surface topography.[39] In the present study, a similar observation was made. To explain if these morphological changes caused the increased MNGCs requires further studies, including in vitro assays.[38]

Surface chemical composition of the ZrO_2 surface might also be responsible for these differences but was not included in this study. Embedding of the blasting material Al_2O_3 on the implant surface[40] can barely serve as a causal explanation, as etching reduces contamination with Al_2O_3.[41] The impact of surface modifications on MNGCs in the present study became significant not before 8 weeks, suggesting a cumulative effect, not an early immediate reaction of the cells to the biomaterial. Moreover, it is obvious from the histological images that the presence of MNGCs is not associated with a local inflammatory reaction or a fibrous encapsulation, thus the presence of MNGCs not necessarily indicated that these cells are "foreign body giant cells." This emphasizes the importance of the effect of the chemical

composition of the ZrO_2 surfaces on the formation of MNGCs. The characteristics of the MNGCs and the process of their genesis is an interesting research topic. The lack of element analysis of the three ZrO_2 surfaces represents a weakness of the present study, but will be considered in future research.

Macrophages might play a role for cells being involved during the bone formation process. The functional spectrum of macrophages is broad and includes catabolic and anabolic processes. While macrophages mediate tissue destruction in situations of chronic inflammation, they also serve as a rich source of growth factors that support wound healing.[28,42] The overall situation is controversial as for example monocyte activation can stimulate an osteogenic response[43] but also inhibit migration, metabolic activity and osteogenic differentiation of human mesenchymal stem cells.[44] In the present study, BIC and MIC were found to be associated, suggesting a possible beneficial effect of the macrophages on bone formation. However, keeping the preliminary status of the data in mind, these findings should be an inspiration to further investigate the possible role of macrophages during the osseointegration process.

CONCLUSIONS

Data from this study suggest that surface modifications of sandblasted ZrO_2 implants can affect bone apposition and the formation of MNGCs. The present data should serve as a scientific basis for the design of future studies.

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REFERENCES

1. Wennerberg A, Albrektsson T. On implant surfaces: a review of current knowledge and opinions. Int J Oral Maxillofac Implants 2010; 25:63–74.
2. Buser D, Mericske-Stern R, Bernard JP, et al. Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multi-center study with 2359 implants. Clin Oral Implants Res 1997; 8:161–172.
3. Meijer HJ, Raghoebar GM, Batenburg RH, Vissink A. Mandibular overdentures supported by two Branemark, IMZ or ITI implants: a ten-year prospective randomized study. J Clin Periodontol 2009; 36:799–806.
4. Jacobs R, Pittayapat P, van Steenberghe D, et al. A split-mouth comparative study up to 16 years of two screw-shaped titanium implant systems. J Clin Periodontol 2010; 37:1119–1127.
5. Buser D, Janner SF, Wittneben JG, Bragger U, Ramseier CA, Salvi GE. 10-year survival and success rates of 511 titanium implants with a sandblasted and acid-etched surface: a retrospective study in 303 partially edentulous patients. Clin Implant Dent Relat Res 2012; 14:839–851.
6. Marinello CP, Meyenberg KH, Zitzmann N, Luthy H, Soom U, Imoberdorf M. Single-tooth replacement: some clinical aspects. J Esthet Dent 1997; 9:169–178.
7. Jung RE, Sailer I, Hammerle CH, Attin T, Schmidlin P. In vitro color changes of soft tissues caused by restorative materials. Int J Periodontics Restorative Dent 2007; 27:251–257.
8. Javed F, Al-Hezaimi K, Almas K, Romanos GE. Is titanium sensitivity associated with allergic reactions in patients with dental implants? A systematic review. Clin Implant Dent Relat Res 2013; 15:47–52.
9. Sandhaus S. [Technic and instrumentation of the implant C.B.S. (Cristalline Bone Screw)]. Inf Odontostomatol 1968; 4:19–24.
10. Pedersen KN. Tissue reaction to submerged ceramic tooth root implants. An experimental study in monkeys. Acta Odontol Scand 1979; 37:347–352.
11. Dubruille JH, Viguier E, Le Naour G, Dubruille MT, Auriol M, Le Charpentier Y. Evaluation of combinations of titanium, zirconia, and alumina implants with 2 bone fillers in the dog. Int J Oral Maxillofac Implants 1999; 14:271–277.
12. Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials 1999; 20:1–25.
13. Warashina H, Sakano S, Kitamura S, et al. Biological reaction to alumina, zirconia, titanium and polyethylene particles implanted onto murine calvaria. Biomaterials 2003; 24:3655–3661.
14. Lacefield WR. Materials characteristics of uncoated/ceramic-coated implant materials. Adv Dent Res 1999; 13: 21–26.
15. Kelly PM, Francis Rose LR. The martensitic transformation in ceramics – its role in transformation toughening. Prog Mater Sci 2002; 47:463–557.
16. Piconi C, Maccauro G, Muratori F, Brach Del Prever E. Alumina and zirconia ceramics in joint replacements. J Appl Biomater Biomech 2003; 1:19–32.

17. Ahmad I. Yttrium-partially stabilized zirconium dioxide posts: an approach to restoring coronally compromised nonvital teeth. Int J Periodontics Restorative Dent 1998; 18:454–465.
18. Schenk RK, Buser D. Osseointegration: a reality. Periodontol 2000 1998; 17:22–35.
19. Schliephake H, Hefti T, Schlottig F, Gedet P, Staedt H. Mechanical anchorage and peri-implant bone formation of surface-modified zirconia in minipigs. J Clin Periodontol 2010; 37:818–828.
20. Hoffmann O, Angelov N, Zafiropoulos GG, Andreana S. Osseointegration of zirconia implants with different surface characteristics: an evaluation in rabbits. Int J Oral Maxillofac Implants 2012; 27:352–358.
21. Gahlert M, Rohling S, Wieland M, Sprecher CM, Kniha H, Milz S. Osseointegration of zirconia and titanium dental implants: a histological and histomorphometrical study in the maxilla of pigs. Clin Oral Implants Res 2009; 20:1247–1253.
22. Gahlert M, Roehling S, Sprecher CM, Kniha H, Milz S, Bormann K. In vivo performance of zirconia and titanium implants: a histomorphometric study in mini pig maxillae. Clin Oral Implants Res 2012; 23:281–286.
23. Rocchietta I, Fontana F, Addis A, Schupbach P, Simion M. Surface-modified zirconia implants: tissue response in rabbits. Clin Oral Implants Res 2009; 20:844–850.
24. Lee J, Sieweke JH, Rodriguez NA, et al. Evaluation of nanotechnology-modified zirconia oral implants: a study in rabbits. J Clin Periodontol 2009; 36:610–617.
25. Kohal RJ, Wolkewitz M, Hinze M, Han JS, Bachle M, Butz F. Biomechanical and histological behavior of zirconia implants: an experiment in the rat. Clin Oral Implants Res 2009; 20:333–339.
26. Saulacic N, Bosshardt DD, Bornstein MM, Berner S, Buser D. Bone apposition to a titanium-zirconium alloy implant, as compared to two other titanium-containing implants. Eur Cell Mater 2012; 23:273–288.
27. Chehroudi B, Ghrebi S, Murakami H, Waterfield JD, Owen G, Brunette DM. Bone formation on rough, but not polished, subcutaneously implanted Ti surfaces is preceded by macrophage accumulation. J Biomed Mater Res A 2010; 93:724–737.
28. Brancato SK, Albina JE. Wound macrophages as key regulators of repair: origin, phenotype, and function. Am J Pathol 2011; 178:19–25.
29. McNally AK, Anderson JM. Macrophage fusion and multinucleated giant cells of inflammation. Adv Exp Med Biol 2011; 713:97–111.
30. Buser D, Broggini N, Wieland M, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004; 83:529–533.
31. Germanier Y, Tosatti S, Broggini N, Textor M, Buser D. Enhanced bone apposition around biofunctionalized sand-blasted and acid-etched titanium implant surfaces. A histomorphometric study in miniature pigs. Clin Oral Implants Res 2006; 17:251–257.
32. Schenk RK, Olah AJ, Hermann W. Preparation of calcified tissue for light microscopy. In: Dickson GR, ed. Methods of calcified tissue preparation. 1st ed. Amsterdam: Elsevier Science Publishers BV, 1984:1–56.
33. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. J Biomed Mater Res 1991; 25:889–902.
34. Cochran DL, Schenk RK, Lussi A, Higginbottom FL, Buser D. Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: a histometric study in the canine mandible. J Biomed Mater Res 1998; 40:1–11.
35. Kohal RJ, Weng D, Bachle M, Strub JR. Loaded custom-made zirconia and titanium implants show similar osseointegration: an animal experiment. J Periodontol 2004; 75: 1262–1268.
36. Stadlinger B, Hennig M, Eckelt U, Kuhlisch E, Mai R. Comparison of zirconia and titanium implants after a short healing period. A pilot study in minipigs. Int J Oral Maxillofac Surg 2010; 39:585–592.
37. Langhoff JD, Voelter K, Scharnweber D, et al. Comparison of chemically and pharmaceutically modified titanium and zirconia implant surfaces in dentistry: a study in sheep. Int J Oral Maxillofac Surg 2008; 37:1125–1132.
38. Refai AK, Textor M, Brunette DM, Waterfield JD. Effect of titanium surface topography on macrophage activation and secretion of proinflammatory cytokines and chemokines. J Biomed Mater Res A 2004; 70:194–205.
39. Gruber R, Hedbom E, Bosshardt DD, Heuberger R, Buser D. Acid and alkali etching of grit blasted zirconia: impact on adhesion and osteogenic differentiation of MG63 cells in vitro. Dent Mater J 2012; 31:1097–1102.
40. Le Guehennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007; 23:844–854.
41. Pypen CM, Plenk HJ, Ebel MF, Svagera R, Wernisch J. Characterization of microblasted and reactive ion etched on the commercially pure metals nobium, tantalum and titanium. J Mater Sci Mater Med 1997; 8:781–784.
42. Wahl SM, Arend WP, Ross R. The effect of complement depletion on wound healing. Am J Pathol 1974; 75: 73–89.
43. Omar OM, Graneli C, Ekstrom K, et al. The stimulation of an osteogenic response by classical monocyte activation. Biomaterials 2011; 32:8190–8204.
44. Chen C, Uludag H, Wang Z, Rezansoff A, Jiang H. Macrophages inhibit migration, metabolic activity and osteogenic differentiation of human mesenchymal stem cells in vitro. Cells Tissues Organs 2012; 195:473–483.