CLINICAL ORAL IMPLANTS RESEARCH

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In vivo evaluation of bioactive glass-based coatings on dental implants in a dog implantation model

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Key words: bioactive glass, bone healing, bone-to-implant contact, dog model, hydroxyapatite, surface modification, titanium implants

Abstract

Objectives: Although titanium is commonly used as a favorable bone implant material due to its mechanical properties, its bioactive and osteoconductive capacity is relatively low. Calcium phosphate ceramics, predominantly hydroxyapatite (HA), have been frequently used for coating purposes to improve the bioactive properties. In view of the suggested osteopromotive capacity of bioactive glasses (BGs), this study aimed to evaluate the effect of BG incorporation into HA coatings on implant performance in terms of bone contact and bone area.

Materials and Methods: A total of 48 screw-type titanium implants with magnetron sputter coatings containing different ratios of HA and BG (HA, $HABG_{Low}$, and $HABG_{High}$; $n = 8$) were placed into the mandible of 16 Beagle dogs. After 4 and 12 weeks, their performance was evaluated histologically and histomorphometrically. Peri-implant bone area percentage (BA%) was determined in three zones (inner, 0–500 μm; middle, 500–1000 μm; and outer, 1000–1500 μm). Additionally, bone-to-implant contact (BIC%) and first bone–implant contact (1st BIC) were assessed for each sample.

Results: After 4 weeks, bone-to-implant contact for the HA- and $HABG_{Low}$-coated groups was significantly higher ($P < 0.05$) than for the $HABG_{High}$ coatings. Mean values for overall BA% showed comparable values for both the $HABG_{Low}$ (58.3%)- and $HABG_{High}$ (56.3%)-coated groups. Data suggest that the relative BA around the HA-coated implants (67.8%) was higher, although this was only significant compared to the $HABG_{High}$ group. After 12 weeks, all three groups showed similar bone-to-implant contact and no differences in BA were found.

Conclusions: The incorporation of BG into HA sputter coatings did not enhance the performance of a dental implant in implantations sites with good bone quality and quantity. On the contrary, coatings containing high concentrations of BG resulted in inferior performance during the early postimplantation healing phase.

Introduction

Oral implants, generally made of pure titanium or titanium-based alloys, are widely used in the prosthetic rehabilitation of fully and partially edentulous patients. Furthermore, multiple long-term clinical studies on implant survival report on high clinical survival rates reaching up to 100% after 5 years in function (Tinsley et al. 2001). The ultimate goal in implant therapy is to achieve an early and strong implant fixation into the native surrounding bone tissue. Although titanium is commonly used as a favorable bone implant material due to its mechanical properties, its bioactive and osteoconductive capacity is relatively low (Le Guéhennec et al. 2007). Therefore, implant surface modification experiments intend to improve the early process of osseointegration, as characterized by an increased bone-to-implant contact and enhanced bone volume in the area surrounding the implant (Fontana et al. 2011). For this purpose, different surface modification approaches have been explored to optimize the interaction between implants

and native bone tissue. By altering either surface topography (i.e., grit blasting and acid etching) or changing the physicochemical properties of the surface (i.e., coating deposition), both the bioactive and osteoconductive properties of the surface can be improved (Wennerberg & Albrektsson 2009).

In view of topographical approaches, it is generally accepted that moderately roughened titanium implants have a superior influence on the bone response in comparison with polished "smooth" implant surfaces (Wennerberg & Albrektsson 2009). Alternatively, physicochemical surface alterations, such as coating deposition with osteopromotive compounds, have been shown to be of special interest in the contemporary field of research (Barrere et al. 2003; Nikolidakis et al. 2006). Calcium phosphate ceramics, predominantly hydroxyapatite (HA), are commonly used for this purpose (Barrere et al. 2003). Multiple short *in vivo* animal studies on calcium phosphate coatings have indicated that the deposition of calcium phosphate onto metal implants enhances early bone remodeling due to the formation of a biological apatite layer that is formed after implant placement (Alexander et al. 2009). Beside CaP ceramics, bioactive glasses (BGs) have been proposed to stimulate bone formation (Pazo et al. 1998; Hench 2006). BGs have been reported to possess superior bioactive properties compared to CaP (Wheeler et al. 2001). Additionally, it has been demonstrated that BGs are not only capable to directly bond to bone (Stanic et al. 2002), but also have an osteopromotive effect on cells due to the formation of a hydrated silica layer and hydroxyl carbonate apatite (HCA) on the glass surface that resembles the inorganic phase of bone (Torricelli et al. 2001). In view of this, Gao et al. (2001) analyzed *in vitro* the effect of silica layers on cell behavior and reported enhanced osteoblast proliferation and differentiation, concluding that bone growth on BG involves stimulatory mechanisms originating from both a chemical and a biological nature. Despite desirable biological characteristics, the use of BGs as a coating material for bone implants has been limited, due to the fact that BG-based coatings show low adhesive properties owing to the lack of chemical bonding between the glass and titanium substrates (Gomez-Vega et al. 2000). In view of this, it has been suggested to codeposit BG and HA to enhance the adhesive properties of the coating (Pazo et al. 1998; Xie et al. 2010). BG can be easily codeposited with HA using radiofrequent (RF) magnetron sputtering, which generates thin, homogenous, well-adherent coatings onto titanium (Wolke et al. 2005). Additionally, deposition via RF magnetron sputtering straightforwardly allows variations in the composition of HABG sputter coatings by only adjusting the individual power on the target materials. Several studies have shown encouraging cell response on these composite coatings *in vitro* (Wolke et al. 2008). However, data on the *in vivo* performance of these coatings remain limited (Xie et al. 2010). In view of this, the aim of this study was to evaluate the biological performance of HABG sputter coatings deposited on commercially available dental implants in a dog mandibular implantation model by histological and histomorphometrical analysis.

Materials and methods

Materials

Forty-eight (48) commercially available cylindrical titanium implants were kindly provided by Biocomp® Industries BV (diameter: 3.4 mm, length: 10 mm; Vught, the Netherlands). The implants featured a 2.0-mm region of microthreads, followed by 2.0-mm conical screw thread, a 2.0-mm smooth region, and a 2.0-mm screw thread close to the apex of the implant. All were grit blasted and acid etched. Before coating deposition, the implants were cleaned ultrasonically in acetone (15 min) and isopropanol (15 min) and thereafter air-dried.

For coating deposition, hydroxyapatite granulate (particle size 0.5–1.0 mm; Cam Bioceramics BV, Leiden, the Netherlands) and BG S53P4 (particle size 90–315 μm; Vivoxid Ltd., Turku, Finland) were used.

Coating procedure

Coating deposition was performed using a commercially available RF magnetron sputter unit (Edwards High Vacuum ESM 100 System, Sussex, UK) as described previously (Wolke et al. 2005). Two materials (i.e., HA and BG) served as simultaneous targets for coating deposition to generate the experimental groups shown in Table 1.

After coating deposition, all implants received an additional heat treatment (HT) for 2 h. The HA coatings were heated at 650°C in an infrared furnace (E4-10-P; Research Inc., Eden Prairie, MN, USA). The composite HABG coatings were heat treated at 550°C in a chamber furnace (UAF; Lenton, Hope Valley, UK). As last step, all implants were sterilized by autoclavation (for 15 min at 121°C) and stored at room temperature.

Coating characterization

The composition of the deposited coatings was determined by Fourier-transform infrared spectroscopy (FTIR, Perkin-Elmer, Waltham, MA, USA) and X-ray diffraction (XRD, Philips θ-20 diffractometer). Average surface roughness values (R_a) and coating thicknesses were analyzed by a Universal Surface Tester (UST; Innowep, Wurzburg, Germany).

Animal model and implantation procedure

Sixteen adult Beagle dogs (1–2 years old, weight 10–12 kg) were used. The research protocol was approved by the ethical committee of King Saud University (Riyadh, Kingdom of Saudi Arabia), and national guidelines for care and use of laboratory animals were observed. The animals were anesthetized, and after intubation, general anesthesia was maintained with Isoflurane® (Rhodia Organique Fine Limited, Avonmouth, Bristol, UK). To reduce peri-operative bleeding, local anesthesia (40 mg/ml xylocain; 5 μg/ml epinephrine) was given. The animals used in this study received mandibular implants from two experimental setups, of which each used on side of the mandible. The outcome of the other experiment is described separately elsewhere.

Extraction phase

Three premolars (P2-4) were delicately removed on the right side of the mandible. First, hemisection of the roots was conducted by drilling a vertical sleeve. After reflection of a full thickness mucoperiostal flap, under direct vision, the roots were removed using elevators and forceps to prevent trauma of the alveolar ridge or labial bone. Intra- and postoperatively, a prophylactic dose of clindamycin (11 mg/kg body weight) was administered for 10 days. Healing time for the extraction sockets was 3 months.

Table 1. **Overview of the experimental groups that were generated after coating deposition by RF magnetron sputtering and additional heat treatment**

Group	Target 1 (power)	Target 2 (power)	Deposition time (h)	Heat treatment (°C)
HA	HA (400 W)	HA (400 W)	2.5	650
$HABG_{Low}$	HA (300 W)	BG (100 W)	7	550
$HABG_{High}$	HA (50 W)	BG (100 W)	20	550

BG, bioactive glass; RF, radiofrequent.

Implantation; time schedule

In the right side of the mandible of the 16 Beagle dogs in total, 48 implants were placed. Each animal received one implant of each experimental group. Implants were placed, according to a rotating randomized schedule, changing the sequence in implant location from mesial to distal for each dog (three implants were placed per dog at the right side of the mandible, resulting in eight implants per group per observation period). Two implantation periods were used, that is, 4 and 12 weeks.

Implantation procedure

Before surgery, the soft tissues were cleaned with a 10% Povidone–iodine. After a midcrestal incision and retraction of the soft tissues, the recipient sites were prepared according to the guidelines provided by the manufacturer (Biocomp® Industries BV). First, three pilot holes (diameter, 2.0 mm; depth, 10 mm) were prepared. Subsequently, the cavity was gradually widened using drills with increasing diameter until the final diameter for implant placement was reached (diameter, 2.8 mm; depth, 10 mm). During low rotational drilling (maximum of 1200 rpm), continuous external irrigation (sterile 0.9% physiological saline) was applied. After preparation, the holes were cleaned and the implants manually placed. Subsequently, cover screws (Biocomp® Industries BV) were placed, and the soft tissues were closed with resorbable sutures (Vicryl® 4-0; Ethicon Products, Amersfoort, the Netherlands; Fig. 1). To reduce postoperative pain, all dogs received a subcutaneous injection with Finadyne® (Schering-Plough, Brussels, Belgium) and a broad spectrum antibiotic (Gentamycin 4 mg/kg body weight) was given intramuscularly for 7 days.

Histological preparation

After 4 and 12 weeks of healing, the dogs were euthanized by an overdose of sodium pentobarbital. The mandibles were removed and put into fixative of 10% neutral buffered formalin solution. Radiographs were made in bucco-lingual direction to identify the exact implant position. All specimens were dehydrated in a graded series of ethanol (70–100%) and eventually embedded in methyl methacrylate. Thin longitudinal sections (10–15 μm) were made in a bucco-lingual direction using a modified sawing microtome technique (Van der Lubbe et al. 1988) and stained with methylene blue/basic fuchsin.

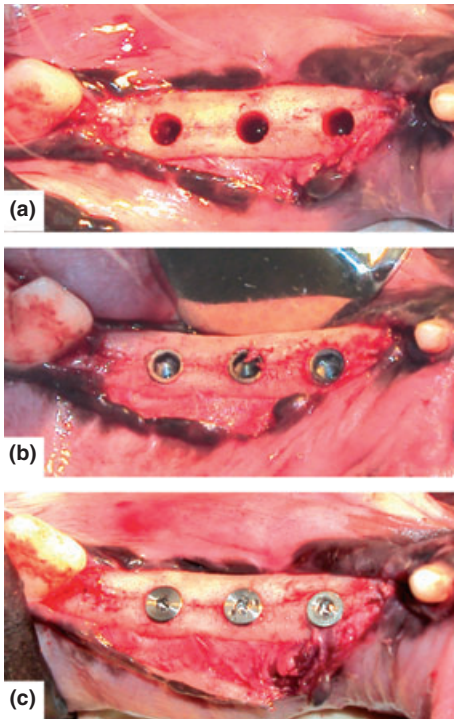

Fig. 1. (a) preparation of osteotomies. (b) The implants installed and (c) closed by cover screws.

Histological and histomorphometrical analyses

Histological evaluation was performed using a Zeis Axio Imager transmission light microscope. Histomorphometry was performed using digital image analysis software (Leica Qwin Pro-image Leica Imaging Systems, Cambridge, UK).

Three quantitative parameters were assessed:

- Percentage of bone-to-implant contact (BIC%). Bone contact was analyzed along the total length of the implant, starting at the first coronal microthread up to the apex of the implant. BIC% was defined as the percentage of the implant surface in direct contact with bone without intervening fibrous tissue layers;
- Percentage of the peri-implant bone area (BA%). The relative BA around the implant was analyzed in a rectangular region of interest (ROI) at the flat part of the implant (Fig. 2). In addition, the ROI was divided into three zones, for which separately the BA% was analyzed: an inner zone (I: 0–500 μm), a middle zone (M: 500–1000 μm), and an outer zone (O: 1000–1500 μm). All measurements were performed for both sides of the implant on three histological sections per implant.
- First bone-to-implant contact (1st BIC): The 1st BIC was defined as the distance between the implant shoulder (without cover screw) and the most coronal bone-to-implant contact (Fig. 3).

Statistical analysis

All measurements were statistically evaluated using GraphPad Instat version 3.10 (GraphPad Software Inc., San Diego, CA, USA). Mean values and standard deviations (SD) were calculated. The method of Kolmogorov and Smirnov was used to confirm that the data were sampled from populations that follow Gaussian distributions. For comparison of data, repeated measurements of one-way analysis of variance (ANOVA) were used with a Tukey's *post hoc* test. Additionally, unpaired t-tests were performed for each experimental group to determine differences between the two implantation periods (4 and 12 weeks). Differences were considered statistically significant at $P < 0.05$.

Results

Coating surface analysis

XRD characterization and FTIR analysis corroborated earlier data by Wolke et al. 2005, showing that all as-sputtered coatings had an amorphous structure. After heat treatment (650°C), only the HA coating altered into a random orientated crystalline apatite structure with specific reflection peaks at $2\theta = 25.9°$, 31,9°, 32.4°, and 34.0°. FTIR analyses showed for all HA- and HABG coatings a cluster from 800 to 1150/cm attributed to the presence of phosphate peaks. Additionally, the HABG coatings showed a cluster from 550 to 600/cm attributed to the presence of silicate peaks (data not shown). Final roughness of the coated implants ranged from R_a = 1.5–2.1 μm (HA = 2.1 μm; $HABG_{Low}$ = 2.0 μm; and $HABG_{High}$ = 1.5 μm). Coating thickness varied for coating type: HA = 0.6 μm; $HABG_{Low}$ = 2.0 μm; and $HABG_{high}$ = 3.0 μm.

Animal experiment

General observations

For all animals, the healing periods after tooth extraction and implant placement were uneventful. The soft tissues around the implants after 4 and 12 weeks did not show any sign of inflammation or adverse tissue reactions. All 48 implants were retrieved. However, three of the implants (one 4-week $HABG_{Low}$ implant, one 12-week $HABG_{High}$ and one 12-week HA) could not be used for evaluation due to implant loosening during histological processing.

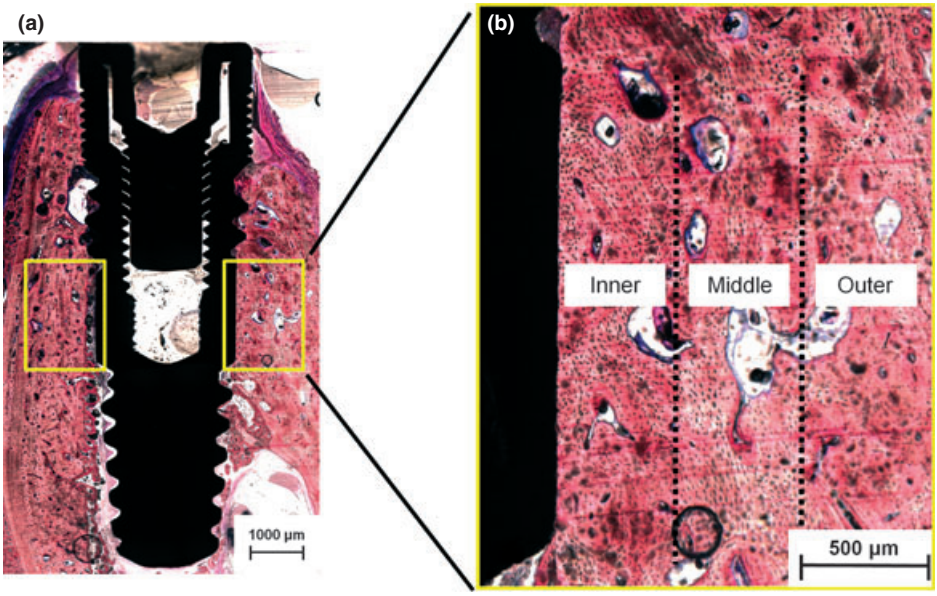

Fig. 2. (a) Schematic representation of the quantitation of relative bone area (BA%) in the region of interest (yellow box) and (b) the division in three different zones: the inner zone (I: 0–500 µm), the middle zone (M: 500–1000 µm), and the outer zone (O: 1000–1500 µm).

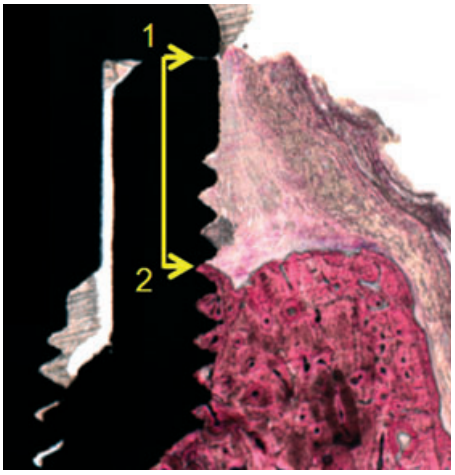

Fig. 3. Schematic representation of the first bone-to-implant contact (1st BIC), defined as the distance from the implant shoulder, without measuring the cover screw (1), to the most coronal bone-to-implant contact (2).

Histological evaluation

A histological representation of the three experimental groups (HA, $HABG_{Low}$, and $HABG_{High}$) after 4 and 12 weeks of healing is shown in Fig. 4.

4-week healing period

Analysis of the histological sections after 4 weeks revealed an intimate contact between implant and surrounding bone without any intervening layers of fibrous tissue (Fig. 5a) for all experimental groups. In more detail, newly formed bone, as characterized by the formation of trabeculae, could be observed on the implant surfaces (Fig. 5d). Although bone formation was present in all experimental groups, the HA-coated implants showed a more uniform and continuous pattern in comparison with the composite HABG groups. Occasionally, crestal bone resorption could be observed, independent of the experimental group. As a result, microthreads at the coronal part of the implants were covered with soft tissues (Fig. 5b). In some of the specimens, the outline of the final drill at the apex of the prepared hole was still visible (Fig. 5c).

12-week healing period

After 12 weeks, maturation of bone surrounding the implants could be observed by replacement of woven bone by lamellar bone as well as by the development of osteons close to the surface of the implant (Fig. 6a). In more detail, ongoing osteoconductive bone formation into the grooves could be observed for most of the HA- and $HABG_{Low}$-coated implants (Fig. 6b). Less pronounced bone formation and maturation had occurred for the $HABG_{High}$ group, especially into the grooves of the implant (Fig. 6c). In some specimens of the latter group, fibrous tissue could be observed along the contour of the implant (Fig. 6d).

Histomorphometrical analysis

4-week healing period

After 4 weeks of healing, BIC% measurements exhibited similar mean overall percentages for the HA- (41.5% ± 19.7) and $HABG_{Low}$-coated implants (45.1% ± 19.3). Mean BIC% for the $HABG_{High}$-coated implants was 29.7% ± 12.5, which was significantly lower ($P < 0.05$) in comparison with both HA- and $HABG_{Low}$-coated groups (Fig. 7).

Mean values for overall BA% showed comparable values for both the $HABG_{Low}$ (58.3% ± 12.2)- and $HABG_{High}$ (56.3% ± 4.0)-coated groups. Data suggest a trend toward a relatively higher amount of bone surrounding HA-coated implants (67.8% ± 0.9), although this was only significant compared to the $HABG_{High}$ group (Fig. 8a). When observing the BA values for the outer, middle, and inner zone for both HABG groups, a decreasing trend in BA% was observed, although this was only statistically significant for the $HABG_{Low}$-coated implants. For the HA-coated implants, the BA% was similar in each zone (Fig. 8b).

Further, 1st BIC measurements showed that after 4 weeks of healing, the distance ranged from 1.34 mm (±0.57) for the HA-coated implants, to 1.76 (±0.89) for the $HABG_{High}$-coated implants. No statistical differences were found between the experimental groups after 4 weeks ($P > 0.05$, Fig. 9).

12-week healing period

After 12 weeks of healing, overall BIC% ranged from 40.5% to 31.1% with no significant differences between the experimental groups. Compared to the 4-week time point, no temporal differences were observed after 12 weeks (Fig. 7).

Means for BA% ranged from 58.2% to 69.4% with no significant differences between the experimental groups compared to the 4-

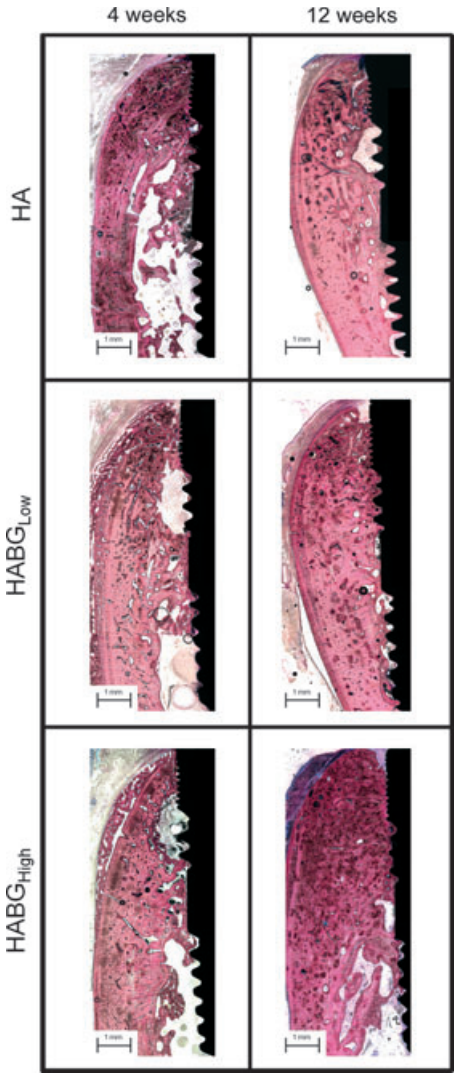

Fig. 4. Histological representation of the three experimental groups (HA, HABG$_{Low}$, and HABG$_{High}$) after 4 and 12 weeks of healing.

week time point. Measurements for the three zones around the implant (i.e., inner, middle, and outer) after 12 weeks revealed no significant differences between the HA and HABG$_{Low}$ groups. However, for the inner and outer zone around the HABG$_{High}$-coated implants, a significant difference ($P < 0.05$) was observed (Fig. 8c).

Data on 1st BIC illustrate that after 12 weeks of healing, no significant differences were found between the experimental groups ($P > 0.05$; Fig. 9).

Discussion

The aim of this *in vivo* study was to evaluate the biological performance of dental implants coated with different ratios of hydroxyapatite (HA) and BG in a dog mandible model. The histological and histomorphometrical analysis after 4 and 12 weeks of implantation demonstrated that, in terms of bone-to-implant contact and peri-implant BA measurements, adding BG to a HA coating failed to improve the biological performance compared to reference HA coating.

When comparing the biological behavior of HA and BG as coatings, the inclusion of an

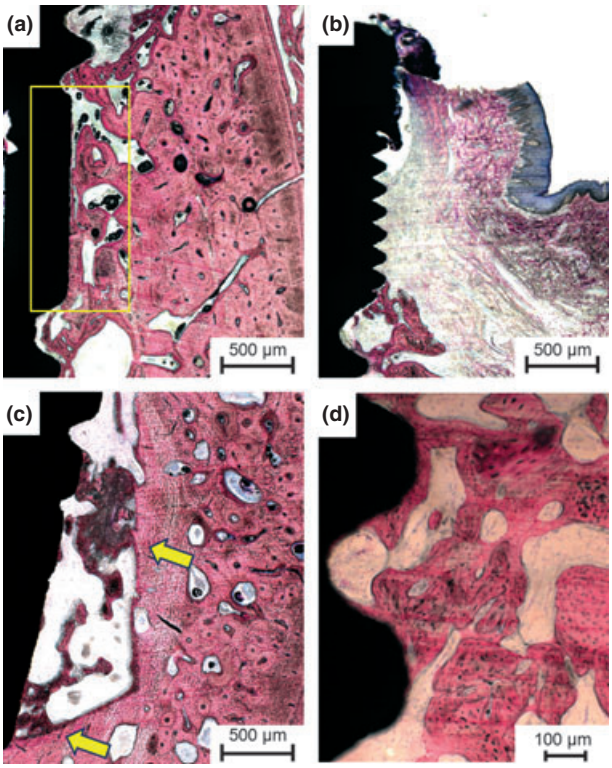

Fig. 5. These images illustrate the histological observations at the 4-week time point for all three experimental groups (HA, HABG$_{Low}$, and HABG$_{High}$). (a) A tight connection with the native surrounding bone and the middle flat part (yellow box) of the implants. (b) Occasional bone resorption at the crestal level of the implant. (c) The outline of the final drill (yellow dashed line) at the tip of the osteotomie. (d) Woven bone close to the implant surface along the contour of the implant.

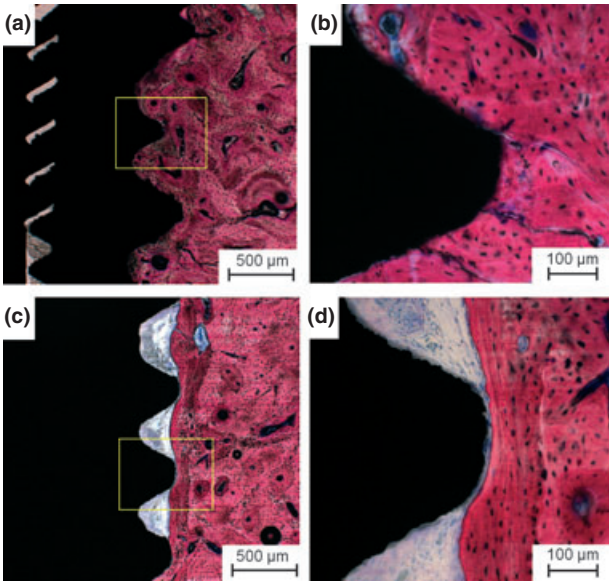

Fig. 6. These images represent the 12-week time point. (a) Prolonged bone formation along the surface for the HA- and HABG$_{Low}$-coated implants. (b) Maturation of bone surrounding the implants with development of osteons close to the surface in a higher magnification for all experimental groups. (c) Invasion of soft tissues along the implant surface, observed for some of the HABG$_{High}$-coated implants. (d) Encapsulation of the implant with aligned fibrous tissues in a higher magnification.

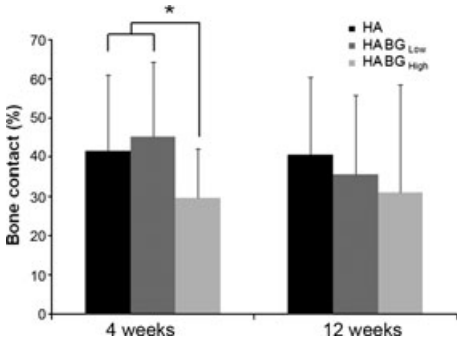

Fig. 7. Results of histomorphometrical and statistical analyses showing the bone-to-implant contact percentage (mean ± SD) after 4 and 12 weeks of healing. *$P < 0.05$.

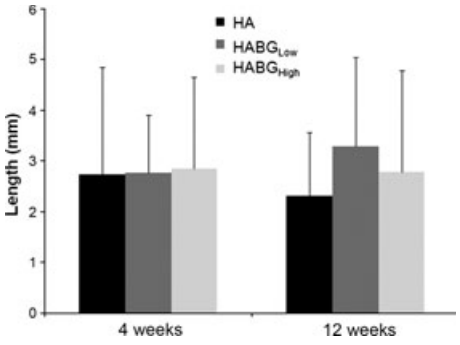

Fig. 9. Results of the histomorphometrical measurements of 1st BIC after 4 and 12 weeks for the three experimental groups (HA, HABG$_{Low}$, and HABG$_{High}$).

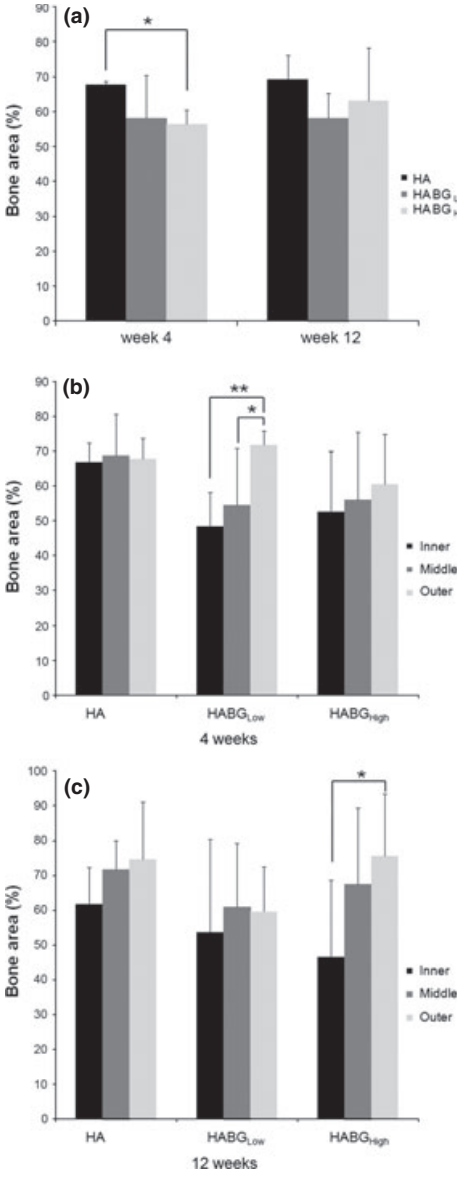

Fig. 8. Results of histomorphometrical and statistical analyses of (a) overall bone area (BA) after 4 and 12 weeks for HA, HABG$_{Low}$, and HABG$_{High}$. (b) BA specified for three zones (i.e., inner, middle, and outer) near the implant surface, after 4 weeks and (c) after 12 weeks. (mean ± SD). *$P < 0.05$; **$P < 0.01$.

experimental group consisting of a pure BG-coated implant is a logical step. However, this was not possible as traditional coating methods have serious limitations as far as BG coating is concerned (Saiz et al. 2002). It is known that weak adhesion of a coated surface can cause delamination or fracture of the coating, leading to an unfavorable *in vivo* response (Ogiso et al. 1998). The weak adherence of BG coatings can be related to the used coating method. During sputtering, the power needs to be relatively low to prevent melting of the BG (100 W vs. 400 W for HA). As a result, the speed of the transported ions is lower, which has an influence on the adhesion of BG to the titanium surface. Additionally, the adhesive strength of the coating is limited due to the absence of a chemical bond between the TiO_2 of the implant surface and the silica (SiO_2) in the BG. Consequently, adhesion of the pure BG coatings mainly depends on the mechanical bonding with the underlying titanium surface roughness, which is created after etching or grit blasting the surface (Gomez-Vega et al. 2000; Wolke et al. 2008). In view of this, it has been suggested to deposit coatings that combine HA and BG as target materials (Pazo et al. 1998). Wolke et al. (2008) analyzed RF magnetron sputtered composite coatings with different compositions of HA and BG *in vitro* and observed that these composite HABG coatings can overcome the adhesive drawbacks of pure BG coatings while maintaining osteogenic properties. In these studies, rapid nucleation of a crystalline apatite phase was found after soaking these samples in simulated body fluid solution. It was stated that the formation of this apatite phase *in vitro* was indicative for the bioactive behavior of these coatings *in vivo*. Although the composition of our HABG coatings was based on these studies, the obtained data of our animal study did not meet these expectations. With respect to BIC% and BA% measurements, no additional effect was observed for the HABG composite coatings in comparison with the pure HA-coated surfaces. In contrast to what was expected, implants with a high amount of BG in the coating (HABG$_{High}$) showed even a significant lower BIC% after 4 weeks.

Implant surface properties play an important role in the early phase of peri-implant osteogenesis. Therefore, surface modification experiments intend to optimize the biological response by tailoring either the surface topography (i.e., grit blasting, acid etching) or chemical properties (i.e., coating deposition) of the implant surface. It is generally accepted that moderately rough surfaces (R_a = ~2 μm) have a superior influence on the bone response in comparison with polished "smooth" implant surfaces (Wennerberg & Albrektsson 2009), due to an increased surface area for cell adhesion and bone formation. Additionally, regarding surface chemistry, calcium phosphate coatings have the potential to improve the early bone response. The beneficial effect of these coatings is ascribed to the great resemblance of the implant surface to the mineral phase of native bone. Still, literature remains inconclusive whether surface topography or chemistry is the decisive parameter in peri-implant bone formation. Gan et al. (2004) conclude from a short-term *in vivo* study on porous sintered titanium structures in the femoral condyle of New Zealand White rabbits that surface chemistry rather than topographical changes enhance peri-implant bone ingrowth. Suh et al. (2007) underline these findings. After 6 weeks of healing, significantly higher BIC% for the CaP-coated implants was observed in comparison with the roughened titanium implants. Fontana et al. (2011), on the other hand, compared titanium implants with a porous oxide or Ca-P-coated surface in a rabbit animal model. They were not able to find a beneficial effect for CaP-coated implants in comparison with the control group in terms of BIC% and mechanical testing (RTQ). On the contrary, the oxidized surface demonstrated higher RTQ values than the Ca-P-coated implants after 2, 4, and 9 weeks of healing. The goal of the present *in vivo* study was to evaluate the effect of incorporating BG into HA coatings. However, it is difficult to change the chemical composition of the implants, without interfering with the surface topography. Implants used for this study were grit blasted and acid etched resulting in R_a values of ~2.3 μm. During the coating process, microporosities

of these roughened titanium surfaces were covered with ions from only the HA or both the HA and BG target. The approximation of similar coating thicknesses was attempted by increasing the deposition time for BG-containing coatings (Table 1), as the power for the BG target is limited to prevent melting. Although this resulted in thin ceramic coatings for all three groups (range 0.6–3.0 μm), the relatively thicker BG-containing coatings apparently decreased the original surface roughness ($HABG_{Low}$ R_a = 2.0 μm; $HABG_{High}$ R_a = 1.5 μm). This decrease in roughness and surface area might have negatively affected the biological performance of BG-containing coatings.

In previous *in vitro* studies (Wolke et al. 2008), it was shown that RF magnetron sputtering is a successful technique to deposit HA and composite HABG coatings with good mechanical properties. However, the elemental composition of the target material changed after sputtering. As such, the weight percentage of SiO_2 in BG decreased from 52.7% to <40% (Wolke et al. 2008). As known from literature, only BG with a 40–60% SiO_2 weight percentage is considered to be osteopromotive (Hench 1998; Saravanapavan et al. 2004). Also, the preferential sputtering of the target material and hence the decrease in concentration SiO_2 below 40% may be the cause of absence of an additional effect on bone healing for both composite HABG coatings.

Another important factor that needs to be considered is the crystallinity of the sputter coatings. Several studies demonstrated that highly crystalline HA coatings have low dissolution rates *in vitro* (Xue et al. 2004) and show high resemblance to the crystallites in native bone (Groot et al. 1998). This can enhance early bone formation (Schliephake et al. 2009b) and can have a positive effect on the differentiation of primary cells to osteoblasts (De Bruijn et al. 1994). X-ray diffraction of our coatings showed that the RF magnetron sputtered HA coatings had a highly orientated crystalline apatite structure after heat treatment. Histomorphometrical analysis in the present study confirmed that these HA coatings can stimulate early bone formation and evoke relatively high BIC% and BA% after both 4 and 12 weeks of implantation. Similar results regarding favorable early bone response around HA-coated implants have been reported by numerous *in vivo* studies (Vercaigne et al. 2000; Nikolidakis et al. 2008). The composite coatings, on the other hand, demonstrated a more amorphous–crystalline structure after RF magnetron sputtering and heat treatment. Although dissolution of the coatings was not analyzed in this study, degradation of the HABG can explain the reduced bone healing (i.e., bone contact and BA) around the HABG-coated implants after 4 and 12 weeks. *In vitro* studies performed by Adams et al., underline that an increase in interfacial ions may lead to cell death and damage newly formed bone (Adams et al. 2001).

The relative amount of bone was measured in three zones around the implant (i.e., inner, middle, and outer). It has been suggested that changes in the inner and middle zone are related to the surgical procedure and the properties of the implant surface, whereas the relative BA in the outer zone reflects the bone density of the implant site (Nikolidakis et al. 2006). After 4 weeks, BA % was similar for the inner, middle, and outer zone around the HA-coated implants, showing that bone formation around the HA-coated implants was present. On the contrary, both HABG coatings had a tendency to have a decreased relative BA toward the inner zone. After 4 weeks, this was significant for the $HABG_{Low}$-coated implants, whereas after 12 weeks, a significant difference between the inner and outer zone was observed for the $HABG_{High}$-coated group. As such, these observations indicate that the surface composition indeed affects bone formation and that this bone formation is inferior for HABG composite coatings compared to HA coatings.

The dog mandible is a suitable and commonly used model for the analysis of surface modifications of titanium bone implants (Schliephake et al. 2009a,b). Histological examination showed bone formation around all implants after 4 and 12 weeks of healing, although occasionally significant loss of marginal bone height around the crestal part of the implants could be observed. The exact reason for this bone loss remains unclear, but might be related to the ratio of implant diameter and the width of the alveolar ridge. Previous studies on marginal tissue reactions after implant placement emphasize that the position of the implant in relation to the buccal bone is of crucial importance (Qahash et al. 2008; Junker et al. 2010) and that the distance between the buccal wall and the implant should be at least 2 mm to maintain the alveolar bone level at the implant platform (Qahash et al. 2008). In our study, however, the majority of the implants were placed within these 2 mm of the buccal bone. Another reason might be the reflection of the soft tissues and periosteum from the alveolar bone (Araujo et al. 2005).

The absence of significant differences after 12 weeks of healing between the experimental groups in terms of BIC% and BA% do not correspond with recent *in vivo* studies by Xie et al. (2010). In these studies, the osseointegration of composite coatings with nano-HA and BG on titanium implants to conventional HA coatings in the femoral condyle of New Zealand rabbits was compared, for which they observed higher BIC% values for the HABG coatings after 12 weeks in comparison with the coatings without the addition of BG. This discrepancy might be related to differences in implantation site and animal species. The dog mandible is a well-established animal model for the evaluation of peri-implant bone healing in clinically comparable conditions (Schliephake et al. 2009a,b). It can be hypothesized that the high bone quality and quantity at the implant site overshadowed a possible significant difference between the experimental group.

Conclusion

Within the limitations of this study, it can be concluded that the incorporation of BG to a HA reference sputter coating does not enhance the biological performance of a dental implant in implantations sites with good bone quality and quantity. On the contrary, coatings containing high concentrations of BG resulted in inferior performance during the early postimplantation healing phase.

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