CLINICAL ORAL IMPLANTS RESEARCH

Hamdan S. Alghamdi
Bart A.J.A. van Oirschot
Ruggero Bosco
Jeroen J.J.P.van den Beucken
Abdullah Al Farraj Aldosari
Sukumaran Anil
John A. Jansen

Biological response to titanium implants coated with nanocrystals calcium phosphate or type 1 collagen in a dog model

Authors' affiliations:
Hamdan S. Alghamdi, Bart A.J.A. van Oirschot, Ruggero Bosco, Jeroen J.J.P.van den Beucken, John A. Jansen, Department of Biomaterials, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
Hamdan S. Alghamdi, Sukumaran Anil, Department of Periodontology and Community Dentistry, College of Dentistry, King Saud University, Riyadh, Saudi Arabia
Abdullah Al Farraj Aldosari, Department of Prosthetic Science, College of Dentistry, King Saud University, Riyadh, Saudi Arabia
Abdullah Al Farraj Aldosari, Sukumaran Anil, John A. Jansen, Dental Implant and Osseointegration Research Chair, King Saud University, Riyadh, Saudi Arabia

Corresponding author:
John A. Jansen
Department of Biomaterials
Radboud University Nijmegen Medical Centre
PO Box 9101, 6500 HB Nijmegen
The Netherlands
Tel.: +31 24 361 4920
Fax: +31 24 361 4657
e-mail: j.jansen@dent.umcn.nl

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Abstract

Objective: The current study aimed to evaluate the osteogenic potential of electrosprayed organic and non-organic surface coatings in a gap-implant model over 4 and 12 weeks of implantation into the dog mandible.

Material and methods: Sixteen Beagle dogs received experimental titanium implants in the mandible 3 months after removal of left premolars (P2, P3 and P4). Three types of implants were installed in each animal: non-coated implant, nano-CaP coated implant and implant with type 1 collagen coating. Both micro-CT and histomorphometry were used to evaluate peri-implant bone response after implantation periods of 4 and 12 weeks. The bone area percentage was assessed histomorphometrically in three different zones (inner: 0–300 μm; middle: 300–600 μm; and outer: 600–1000 μm) around the implant surface. Bone-bridging of the gap was also calculated for each sample.

Results: Four weeks after implantation, nano-CaP and collagen-coated implants showed significantly higher bone volume (BV) in the inner zone compared with non-coated implants ($P < 0.05$ and $P < 0.01$). After 12 weeks, histomorphometric analysis showed comparable amounts of BV between all experimental groups. Also, no significant difference was found in the BV, as measured using micro-CT, between the implant groups. Absolute bone ingrowth measurements were highest for collagen-coated implants, but these differences were not significant.

Conclusion: The obtained data failed to provide a consistent favourable effect on bone formation of the collagen coating over 3 months of implantation. It is concluded that the source of the collagen as well as the limited osseous environment overshadowed a possible effect of the applied implant surface modifications. Similarly, the tested nano-apatite surface coating did not improve peri-implant bone ingrowth into a gap-implant model.

The concept of osseointegration describes the healing process at the implant–bone interface. Subsequently, implant surface modification experiments intend to improve the properties of the implant surface and to encourage the bone healing response (Puleo & Thomas 2006; de Jonge et al. 2008). The applied surface modifications are physical or chemical alterations or a combination thereof. The final goal of the surface modification is to make the implant surface more osteophilic, i.e. attractive for bone forming cells (de Jonge et al. 2008; Mendonca et al. 2008).

Calcium phosphate (CaP) coatings are known to promote *in vitro* cell attachment and the production of extracellular matrix (ECM), whereas *in vivo* studies confirmed the increased osteoconductive properties of CaP coating in comparison to non-coated implant (Siebers et al. 2004, 2007). This favourable property of CaP coatings is supposed to be due to the similarity in chemical composition between synthetic CaP and CaP as present in natural bone. Despite this chemical similarity, the deposited coatings do not show structural or biological similarity with bone tissue. Bone is not only composed of the inorganic CaP phase but also includes an organic matrix, i.e. collagen and non-collagenous proteins. Therefore, currently, new techniques, such as electrostatic

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spray deposition (ESD), are explored to provide implants with surface coatings that mimic the inorganic as well as organic components of living bone (Leeuwenburgh et al. 2003; de Jonge et al. 2008). The organic part of the bone ECM is composed of collagen type 1 fibrils embedded in an amorphous substance, which consists of glycosaminoglycans and various bone proteins. The ECM components participate actively in the regulation of cellular processes and responses. Therefore, implant surface modifications with components of bone ECM appears attractive to modulate specific intrinsic osteogenesis directly at the bone–implant interface (Stadlinger et al. 2007; de Jonge et al. 2008). The ECM works as a scaffold for bone forming cells and influences migration, adhesion and differentiation of these cells (Geissler et al. 2000; de Jonge et al. 2010). So far, only a limited number of ECM molecules have been successfully deposited on an implant surface (Scharnweber et al. 2004). For instance, collagen type 1, the major structural protein in ECM, has been used as an organic implant coating material. Recent studies have demonstrated the effective role of a collagen coating in stimulating cellular responses, increasing bone growth and improving bone-to-implant contact (Rammelt et al. 2006; Stadlinger et al. 2008c; Schliephake et al. 2009; de Jonge et al. 2010).

To date, few research labs succeeded to deposit homogeneous inorganic and/or organic coatings onto titanium implants. In previous *in vitro* experiments (de Jonge et al. 2009), the electrospray process was already used to deposit nano-CaP, collagen and alkaline phosphatase (ALP) coatings on titanium surfaces to improve the adhesion of osteoblast-like cells and to enhance their mineralization. The studies confirmed that these newly developed coatings are promising for an early and direct apposition of bone mineral to the implant surface. Furthermore, in a small animal model, thin CaP/ALP composite coatings demonstrated to accelerate early bone formation starting from the implant surface (Schouten et al. 2009). The next step for evaluating the potential of organic and/or inorganic coatings involves *in vivo* implantation in an established preclinical animal model. Considering the suggested need for dental implants with improved osseointegration, it is necessary that newly designed implant surfaces are not only assayed under optimal experimental conditions, but rather, are also under challenging clinical conditions. For example, in the clinical situation, gaps between an implant and bone will arise during surgery and as a result of anatomical variation in healthy as well as compromised bone. The fit of the implant in the drilled implant bed can influence the final bone-to-implant contact. Carlsson et al. (1988) proved already that the critical gap between bone and a cylindrical titanium implant that prevents direct bone apposition on the implant is close to zero.

In view of this, the aim of the present study was to evaluate the biological performance of electrosprayed nanocrystals CaP and collagen coatings *in vivo* to determine to what extent these coatings can improve the osteogenic potential of the implant surface in a 1 mm gap model during implantation periods from 4 to 12 weeks.

Materials and methods

Implants

Cylindrically shaped implants (diameter: 3.2 mm; length: 8 mm) provided with a radial gap (1 mm) were made of commercially pure titanium (Fig. 1a).

All implants were cleaned ultrasonically in nitric acid 10% (15 min), acetone (15 min), and ethanol (15 min) successively and thereafter air dried. Then, implants were left as-prepared or provided with two types of ESD coating.

Coating deposition

Electrostatic spray deposition coatings were deposited using the process previously described by de Jonge et al. (2010). The following standardized conditions were applied: 15% relative humidity; 30°C substrate holder temperature; 40 mm nozzle-to-substrate distance; 0.15 ml h^{-1} liquid flow rate; and 8–10.5 kV applied voltage. For deposition of nano-CaP coatings, nano-sized crystalline carbonate apatite particles (Berkely Advanced Biomaterials Inc., San Leandro, CA, USA) were diluted in a 10 : 90 vol.% ethanol : ddH2O solution prior to electrospraying. To deposit the collagen coatings, commercially available rat tail collagen type 1 (BD Biosciences, Sparks, MD, USA) was used. Coating deposition was done in three separate runs (with in between implant turning of 120°) of 30 min each to obtain complete coating coverage. Only the middle (gap) part of the implants was coated. Top and apical portion of the implants were used to provide their initial stability and did not receive surface modification. All coated implants were stored at −20°C, after which lyophilization was applied. The non-coated implants were autoclaved before implantation.

Animal model and surgical procedures

The animal protocol was approved by the animal ethical committee of King Saud University, College of Dentistry, Riyadh, Saudi Arabia and national guidelines for care, and use of laboratory animals were obeyed.

A total of 48 implants ($n = 8$ for each experimental group at each implantation period) were inserted in the mandible of 16 Beagle dogs (1–2 years old and weighing 10–15 kg) for a period of 4 and 12 weeks. The dogs first underwent extraction of left mandibular premolars (P2, P3 and P4) and the extraction sockets were allowed to heal for 3 months. Thereafter, implants were

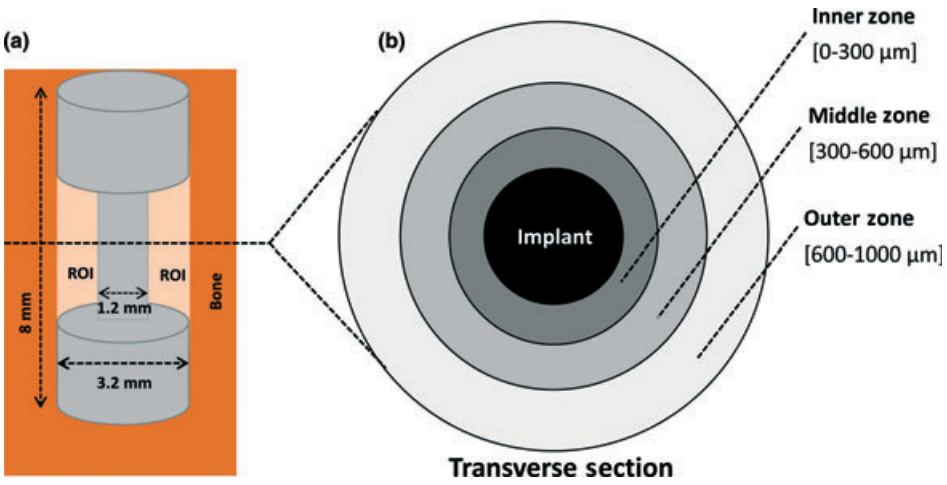

Fig. 1. Schematic drawing of the implant design and the preparation of the histological transverse sections. a) The amount of bone volume (BV) in the gap was determined by setting of a region of interest (ROI). b) For each individual cross-sectional sample, a set of three different zones were used for histomorphometrical analysis. Inner, middle and outer zones were marked as circles, starting at implant surface with distance of 0–300 µm, 0–600 µm and 0–1000 µm.

installed ($n = 3$ per animal, i.e. nano-CaP, collagen and non-coated).

Extraction procedure

Teeth were extracted under general anaesthesia. An intramuscular (IM) injection of ketamine hydrochloride (5 mg/kg) and diazepam (1 mg/kg) was used to sedate the animals before the procedure. The oral tissues were disinfected with a 10% Povidone-iodine. Then, local anaesthesia (lidocaine 2% with 1 : 100,000 epinephrine) was injected around the lower premolars. Following complete anaesthesia, three lower premolars (P2, P3 and P4) in the left side were extracted atraumatically. After reflection of full-thickness mucoperiosteal flaps, the roots were separated using a high-speed diamond bur with saline coolant. Thin elevator and forceps were used to luxate and remove the separated roots gently. Flaps were closed with resorbable sutures (Vicryl 4/0 sutures). Gentamycin (4 mg/kg) was administered intramuscularly for 7 days.

Implantation procedure

After a healing period of 3 months, implants were installed. Before surgery, the dogs were sedated, and local anaesthesia was injected in the field. Subsequently, an incision was made at the bone crest, and a mucoperiosteal flap was reflected on both ridge sides (buccal and lingual). Implant sites were prepared using a low-speed drill series with saline irrigation. Final drill diameter was 3.2 mm. Thereafter, implants were inserted manually below the crestal bone level. To ensure complete randomization, the implants were placed according to a rotating design, in which the position of each implant shifted up one position compared with the previous dog. Finally, the flaps were closed using Vicryl (4/0) sutures to achieve primary soft tissue closure. Gentamycin (4 mg/kg) was administered intramuscularly for 7 days. The dogs were kept on a soft diet for 2 weeks after the surgical procedure.

Analysis

After implantation periods of 4 and 12 weeks, the animals were euthanized via an overdose of sodium pentobarbital (20 mg/kg). The mandibles including the implants were harvested and immediately fixed in 10% neutral buffered formalin solution after removal of excess tissue. Using a diamond circular saw, the samples were divided into smaller specimens suitable for micro-CT scanning and histological processing.

Micro-computed tomography

Prior to scanning, bone blocks containing only one implant each, were dehydrated in ethanol 70% and wrapped in Parafilm (SERVA Electrophoresis GmbH, Heidelberg, Germany) to prevent drying during scanning. For a quantitative 3D analysis, the specimens were placed vertically onto the sample holder of a Skyscan 1072 desktop X-ray Micro-computer tomography (micro-CT) system (Skyscan, Kontich, Belgium), with the long axis of the implant perpendicular to the scanning beam. Subsequently, a high resolution scan was recorded at a 30 μm voxel resolution. Then, using Nrecon V1.4 (Skyscan), a cone beam reconstruction was performed on the projected files. Thereafter, a constant region of interest (ROI) was set along the length of the implant gap, using CTAn V1.8 (Skyscan). The ROI included the complete gap area surrounding the implant core (total standardized distance of interest of 3000 μm). Finally, for all images, a threshold was manually selected to isolate bone tissue and to preserve its morphology, while excluding the implant material. Per implant, the parameters of bone volume (BV) and tissue volume were measured, after which the amount of bone volume was calculated.

Histological preparations

Subsequent to micro-CT scanning, the specimens were dehydrated in a graded series of ethanol (70–100%), washed with acetone, and embedded in methyl methacrylate (MMA). After polymerization, non-decalcified thin sections (~10 μm) were prepared (at least three of each implant), using a modified sawing microtome technique (van der Lubbe et al. 1988) and stained with methylene blue and basic fuchsin. Cross-sections were made perpendicular to the long axis of the implant.

Histomorphometrical evaluation

To evaluate the bone response in the gap around the implants, histological evaluation was carried out using a light microscope (Axio Imager Microscope Z1, Carl Zeiss Micro Imaging GmbH, Göttingen, Germany). Histomorphometrical analysis was performed using a computer-based image analysis technique (Leica Qwin Pro-image analysis software; Leica Imaging Systems, Cambridge, UK). Quantitative measurements were performed for three histological sections per implant (at magnification 25×). The average of these measurements was used for statistical analysis. One of the quantitative parameters as assessed was the peri-implant bone volume in the gap. Therefore, the amount of bone area was determined by setting of a ROI for each individual sample section. This ROI was individually set by determining the peripheries of the gap (the original margins of the drill hole) and placing a circle (Fig. 1a, b). To determine the amount of BV, three different circular zones were defined starting at the implant surface, i.e. inner (0–300 μm), middle (300–600 μm) and outer (600–1000 μm (Fig. 1b). Per section, the amount of bone area per zone was calculated as the area percentage of bone inside the circle.

Bone-bridging of the gap was also calculated for each section. Using Qwin software, 180 lines were automatically drawn 360° around the implant surface. Each line started from the implant surface and stopped when bone was contacted. The lengths of lines were displayed in millimetres and indicated the distance between implant surface and bone. As the original width of the gap was known, the obtained data were used to estimate the average bone ingrowth distance for each sample (Fig. 2).

Statistical analysis

For statistical analysis, SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used. Paired and unpaired *t*-tests were used to evaluate the effects of the implant surface modifications on the peri-implant bone volume and bone ingrowth distance at 4 and 12 weeks of implantation. For each statistical comparison, the model (paired vs. unpaired *t*-test) that showed the most precise outcomes (with narrowest width of the 95% confidence interval of the difference) was only considered.

Fig. 2. Method of measuring bone-bridging-gap was performed using specific software. A total of 180 green lines were automatically drawn from the surface of the implant. Each line stopped when bone was hit. As the width of the gap and the lengths of lines were known, the obtained data were used to estimate the average bone ingrowth distance for each sample.

Table 1. **Summary of number of implants placed and retrieved for the study analyses**

	No. of implants placed	No. of implants retrieved
4 weeks		
Nano-CaP	8	7*
Collagen	8	6†
Non-coated	8	7*
12 weeks		
Nano-CaP	8	8
Collagen	8	6†
Non-coated	8	8

*One implant fell out during wound healing period
†Two implants fell out during wound healing period

Statistical comparisons of bone volume between all implant types were also performed for three different zones (inner, middle, outer) around each implants. Differences were considered significant at probability (P) values smaller than 0.05.

Results

All animals remained in good health during the experimental period and did not show any postoperative wound healing complications. At sacrifice, no signs of inflammation or adverse tissue reaction were seen around the implants. Table 1 depicts the number of implants placed and retrieved after implantation. Of the 48 installed implants, a total of 42 implants could be retrieved. In 4-weeks group, four implants were lost (1 non-coated, 1 nano-CaP-coated and 2 collagen-coated), whereas in 12-weeks group, only two collagen-coated implants were lost.

Descriptive histological evaluation

Light microscopic examination demonstrated that generally all sections showed bone apposition and ingrowth of newly formed bone into the gap around the implants (Fig. 3). At both implantation times, the margins of the original drill hole were still visible, and no inflammatory reactions were observed in any of the specimens. Bone remodelling activity was observed inside all implant gaps, irrespective of the implant surface modification.

The cross-sections showed an apparent histological difference in bone response and adaptation to the three different implant surfaces. At 4 weeks, histological sections revealed that the bone tissue was never in tight contact with the implant surface, but a fibrous tissue layer of varying thickness was interposed between the implant and bone. For the surface-coated implants (nano-CaP and collagen), the bone present in the gap appeared to have grown closer to the implant surface. For the non-coated implants, the intervening fibrous layer was always apparently thicker. After 12 weeks, an evident increase of bone ingrowth had occurred for all implants compared with 4 weeks of implantation with compact lamellar bone filling most of the gap area. Bone ingrowth had also proceeded into close proximity of all the implant surfaces. Nevertheless, direct bone contact with the implant surface was never observed, and a fibrous tissue layer was still interposed between the bone tissue and implant surface.

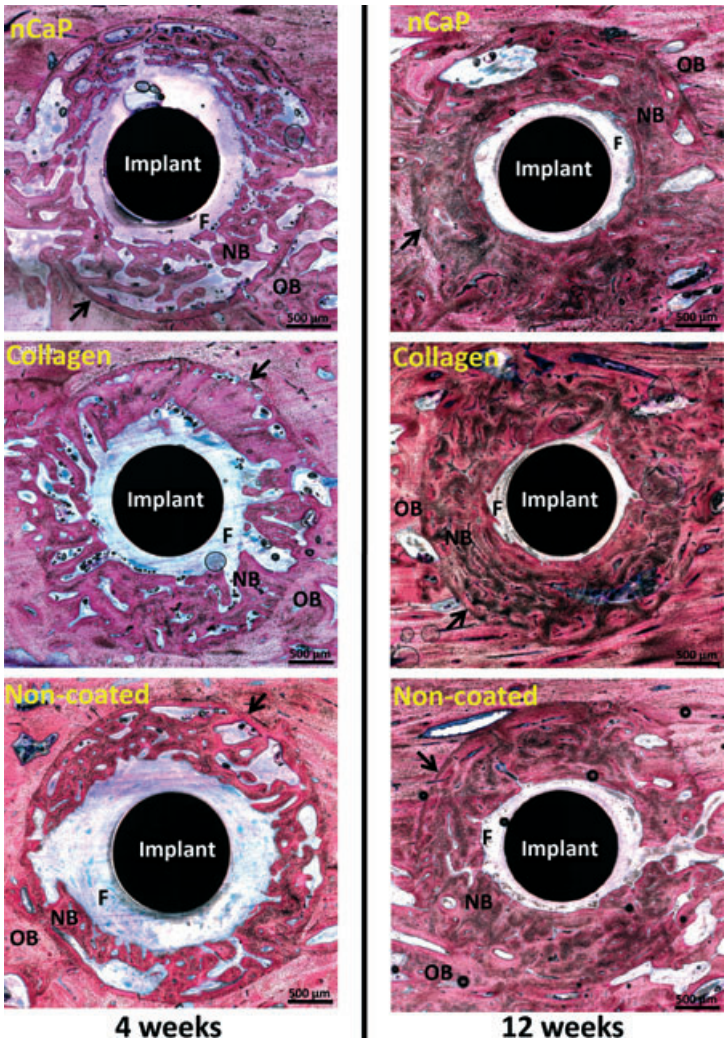

Fig. 3. Transverse histological images were obtained for each surface modification at 4 and 12 weeks. The margins of the original drill hole (arrows) were still visible between new bone (NB) and old bone (OB). Fibrous tissue (F) was always interposed between the implant and bone. At 4 weeks, the bone front appeared to be closer to the coated surfaces. Nano-CaP implants showed a higher number of marrow spaces compared with the other implants. After 12 weeks, regions of lamellar compaction and a high histological bone density were with just a few marrow spaces adjacent to all implant surfaces.

Micro-CT analysis

For all experimental groups, mean data regarding bone volume measurements at 4 and 12 weeks are listed in Table 2. Although the absolute mean value for bone volume at 4 and 12 weeks post-implantation was higher for the collagen-coated implants, statistical testing revealed that the observed difference was not significant ($P > 0.05$).

Histomorphometrical analysis

Mean data and the outcome of statistical analyses regarding bone area percentage and gap healing measurements for the experimental groups at 4 and 12 weeks are presented in Tables 3 and 4 and Figs 4 and 5.

Bone volume (%)

Regarding overall bone volume, significant differences were observed only between the collagen (61.4%) and non-coated (47.5%) groups at 4 weeks ($P < 0.05$; Table 3). In addition, the overall bone volume values for only the nano-CaP ($P = 0.010$) and non-coated

Table 2. **Micro-CT data and the outcome of statistical analyses regarding bone volume (%) for all implant surface groups at 4 and 12 weeks**

			t-test*		
	Mean ± SD		Model	MD [95% CI]	*P* value
4 weeks					
Nano-CaP	45.7 ± 6.9	Nano-CaP vs. Collagen	UP	−7.5 [−16.3, 1.3]	0.087
Collagen	53.2 ± 7.5	Nano-CaP vs. Non-coated	P	−2.1 [−10.4, 6.3]	0.569
Non-coated	47.7 ± 10.7	Collagen vs. Non-coated	P	2.0 [−5.8, 9.8]	0.542
12 weeks					
Nano-CaP	45.9 ± 9.9	Nano-CaP vs. Collagen	UP	−10.0 [−21.6, 1.6]	0.084
Collagen	55.9 ± 9.7	Nano-CaP vs. Non-coated	P	−4.4 [−11.7, 2.8]	0.184
Non-coated	49.4 ± 12.2	Collagen vs. Non-coated	P	2.2 [−6.8, 11.3]	0.553

*Paired (P) and unpaired (UP) *t*-test models were performed. Mean deference (MD), 95% confidence interval (CI), and *P* value were presented for the most precise model (with narrowest width of CI)

Table 3. **Histomorphometrical data and the outcome of statistical analyses regarding overall bone volume (%) between the various implant surface groups at 4 and 12 weeks**

			t-test*		
	Mean ± SD		Model	MD [95% CI]	*P* value
4 weeks					
Nano-CaP	49.5 ± 11.8	Nano-CaP vs. Collagen	P	−7.5 [−16.2, 1.2]	0.077
Collagen	61.4 ± 9.7	Nano-CaP vs. Non-coated	UP	2.1 [−11.2, 15.3]	0.741
Non-coated	47.5 ± 11.0	Collagen vs. Non-coated	UP	13.9 [1.2, 26.7]	0.035†
12 weeks					
Nano-CaP	67.2 ± 10.9	Nano-CaP vs. Collagen	UP	−5.6 [−18.9, 7.7]	0.380
Collagen	72.7 ± 11.9	Nano-CaP vs. Non-coated	P	0.8 [−8.37, 10.0]	0.833
Non-coated	65.1 ± 10.9	Collagen vs. Non-coated	UP	7.6 [−5.7, 21.0]	0.235

*Paired (P) and unpaired (UP) *t*-test models were performed. Mean deference (MD), 95% confidence interval (CI), and *P* value were presented for the most precise model (with narrowest width of CI)
†indicate significant difference ($P < 0.05$)

Table 4. **Bone volume (%) and statistical analyses for inner, middle and outer zones between the various implant surface groups at 4 and 12 weeks**

			t-test†		
	Mean ± SD		Model	MD [95% CI]	*P* value
Inner zone					
4weeks					
Nano-CaP	23.1 ± 11.8	Nano-CaP vs. Collagen	P	−4.0 [−15.6, 7.7]	0.424
Collagen	28.4 ± 10.1	Nano-CaP vs. Non-coated	UP	12.6 [1.9, 23.4]	0.025*
Non-coated	10.5 ± 5.6	Collagen vs. Non-coated	UP	18.0 [7.1, 28.7]	0.005**
12weeks					
Nano-CaP	38.2 ± 15.7	Nano-CaP vs. Collagen	UP	−14.2 [−32.2, 3.8]	0.111
Collagen	52.4 ± 14.7	Nano-CaP vs. Non-coated	UP	0.7 [−17.2, 18.6]	0.933
Non-coated	37.5 ± 17.6	Collagen vs. Non-coated	P	16.7 [−1.1, 34.5]	0.060
Middle zone					
4weeks					
Nano-CaP	57.1 ± 18.3	Nano-CaP vs. Collagen	UP	−17.4 [−37.6, 2.8]	0.085
Collagen	74.5 ± 14.1	Nano-CaP vs. Non-coated	UP	−3.4 [−23.7, 16.8]	0.717
Non-coated	60.5 ± 16.4	Collagen vs. Non-coated	UP	13.9 [−4.9, 32.8]	0.132
12weeks					
Nano-CaP	81.5 ± 12.8	Nano-CaP vs. Collagen	UP	1.3 [−14.2, 16.8]	0.860
Collagen	80.2 ± 13.6	Nano-CaP vs. Non-coated	UP	3.3 [−9.4, 16.0]	0.590
Non-coated	78.3 ± 10.8	Collagen vs. Non-coated	UP	2.0 [−12.2, 16.1]	0.765
Outer zone					
4weeks					
Nano-CaP	69.1 ± 17.0	Nano-CaP vs. Collagen	UP	−13.1 [−32.2, 6.0]	0.159
Collagen	82.3 ± 13.8	Nano-CaP vs. Non-coated	UP	−3.4 [−20.3, 13.5]	0.670
Non-coated	72.5 ± 11.6	Collagen vs. Non-coated	UP	9.7 [−5.8, 25.2]	0.195
12weeks					
Nano-CaP	82.1 ± 11.1	Nano-CaP vs. Collagen	UP	−3.0 [−16.2, 10.1]	0.625
Collagen	85.2 ± 11.4	Nano-CaP vs. Non-coated	P	7.3 [−1.3, 15.8]	0.082
Non-coated	77.3 ± 10.3	Collagen vs. Non-coated	UP	7.8 [−4.9, 20.5]	0.203

†Paired (P) and unpaired (UP) *t*-test models were performed. Mean deference (MD), 95% confidence interval (CI) and *P* value were presented for the most precise model (with narrowest width of CI)
*indicates significant difference ($P < 0.05$)
**indicates significant difference ($P < 0.01$)

($P = 0.008$) groups showed significant differences between the 4 and 12 weeks time point (Fig. 4).

For the different peri-implant zones (inner, middle and outer), nano-CaP as well as collagen-coated implants showed at 4 weeks of implantation a significantly higher bone volume in the inner zone compared with non-coated implants ($P < 0.05$ and $P < 0.01$; Table 4). For collagen-coated implants, the absolute bone volume values were highest in the middle and outer zone, but the differences were not statistically significant (Table 4). After 12 weeks of implantation, bone formation increased significantly for collagen ($P = 0.008$) and non-coated implants ($P = 0.002$) in the inner zone compared with 4 weeks as well as for nano-CaP ($P = 0.009$) and non-coated implants ($P = 0.026$) in the middle zone. However, further statistical analysis of the 12 weeks data revealed comparable amounts of bone volume in the various zones between all implant groups.

Implant-gap healing

The gap bridging data, as presented in Fig. 5, confirmed the bone volume measurements. At 4 and 12 weeks of implantation, the absolute average values for bone ingrowth distance were highest for collagen-coated implants, but these differences were not significant ($P > 0.05$).

Discussion

The current study aimed to evaluate the osteogenic effect of two implant surface coatings (nano-CaP and collagen) after 4 and 12 weeks, using a so-called implant-gap model with non-coated implants as controls. The results suggested that the collagen-coated implants seemed to have a favourable effect on bone formation inside the gap, but the observed difference was not consistently significant.

Six implants were lost during the 3 months evaluation period, which is likely due to the design of the used implants, i.e. cylindrical and non-threaded. In the current study, the site preparation was the same as the implant diameter, and a good fit was achieved for all implants due to the high-density bone of the dog mandible. However, it has to be emphasized that the implants were not provided with screw-threads and had only an initial stability at their apical and crestal side. Therefore, their initial stability is not suited for the chewing forces that usually result in serious loading even for the edentulous man-

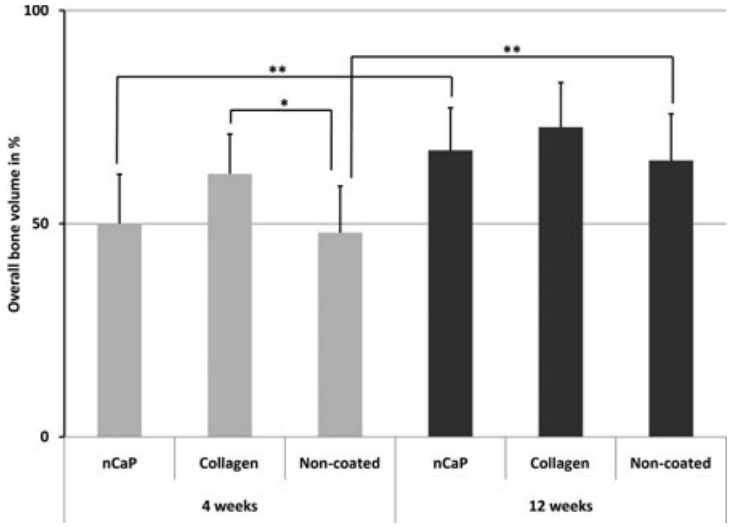

Fig. 4. Overall bone volume and statistical analysis between the various experimental groups at 4 and 12 weeks. (*) indicates significant difference is $P < 0.05$. (**) indicates significant difference is $P < 0.01$.

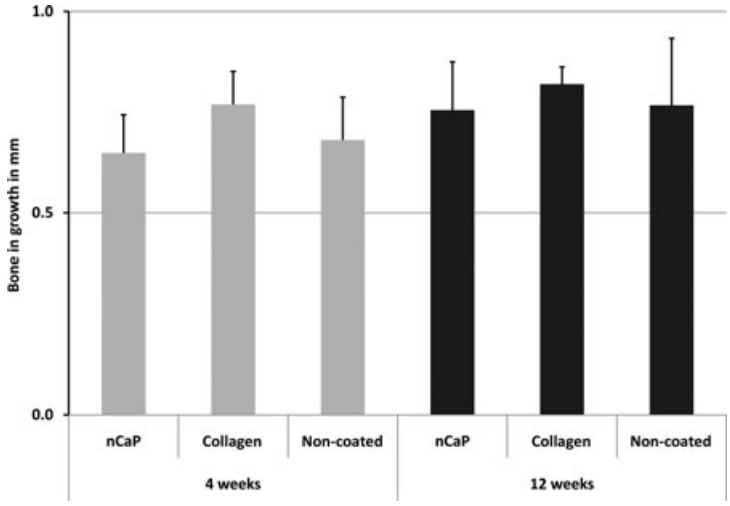

Fig. 5. Representation of bone ingrowth measurements respective to the various implant surface coatings after 4 and 12 weeks.

dible (Lin et al. 1992). This is confirmed by the position of the lost implants, as most of them were situated in the middle position, which is more prone to insult due to chewing forces, while the mesial and distal implants are protected by the neighbouring natural teeth. In addition, the drilling procedure to create the implant bed is always accompanied by bone damage, which is known to occur till a distance of about 1 mm from the original drill walls. Bone damage is associated with necrosis of bone, which may decrease the fixation of non-threaded implants during initial healing (Ooms et al. 2003).

It has to be noticed that the implant surfaces were left non-coated or provided with just a collagen or nano-CaP coating. Implants were not provided with a composite coating composed of collagen and nano-CaP. Although use of such composite coatings was described for *in vitro* studies using titanium disks (de Jonge et al. 2010), the current implant design and coating set-up did not allow the deposition of such composite coatings.

Implant-gap healing model

The histological evaluation clearly showed that bone was extending from the pre-existent surrounding bone into the implant gap. However, bone was never seen in direct contact with the three different implant surfaces, and fibrous tissue was always interposed between the implant surface and the newly formed bone. Evidently, the applied coatings were not able to allow complete bridging of the created 1 mm wide gap. Compared with other studies (Clemens et al. 1997, 1998; Manders et al. 2006) that also used a gap model, it has to be concluded that the deposited coatings in the current composition lack the appropriate osteogenic properties to enable complete gap closure. Still, it has to be emphasized that differences existed between the current study and the previously performed studies. For example, the implants in the earlier studies were installed into the goat femoral condyle, while we inserted the implants in the dog mandible. Craniofacial bone is described to evolve into a different implant bone healing compared with that in long bones (Roberts 1988).

The lack of effect of complete gap closure for the coated implants can also be caused by the design of the implants as explained before. The effect of loss of implant fixation will be more deleterious in the oral cavity compared with implants installed in the long bones. The ingress and chewing of food will always result in serious loading of both the mandibular bone and the installed implants. Implant movement during the initial healing phase has an unfavourable effect on the osseointegration of oral implants (Ooms et al. 2003). Perhaps, this effect was even enhanced because the currently used implants had only an initial bone contact at their apical and crestal side. This effect could have been more enhanced because the implants used in some of the previous studies had a somewhat different design. For example, Clemens et al. (1997) used separate cylindrical titanium plugs with spacers on both endings to ensure sufficient implant stability, whereas Manders et al. (2006) manufactured an implant, in which the gap area was composed of a flat surface and still partly 100% initial bone contact existed over the complete length of the implant. As a consequence, not only would the stability of those implants have been higher compared with the ones of the present study but also, conduction of bone over the implant surface into the gap area would be increased.

Recent studies of Jung et al. (2007) and Lai et al. (2009) indicated that bone-to-implant contact can be achieved even when gaps of up to 2 mm are present between the pristine bone and the implant surface. The current study could not confirm these results, which can be due to the different study design. Jung et al. (2007) and Lai et al. (2009) created circumferential coronal defects around the implants, whereas in the present study, gaps were created within the implants design. Accordingly, the coronal defects are expected to have superior bone apposition, because the

healing enhanced from the lateral and apical bone walls of the defects (Botticelli et al. 2003). However, the bone healing in the current study was only achieved form the lateral bone side.

Finally, this study showed no satisfactory bone-bridging of the gap after a 12-week healing period. Therefore, it could be assumed that an extended follow-up was needed.

Collagen coating for titanium implants

The biological benefits of type 1 collagen, the major ECM protein, on bone regeneration are well recognized (de Jonge et al. 2008). An *in vitro* study by de Jonge et al. (2010) showed that electrosprayed collagen deposition on titanium discs stimulated the osteogenic behaviour of bone marrow stromal cells (MSCs). The interaction of the MSCs with the collagen coating stimulated alkaline phosphatase activity and increased mineral deposition. In addition, type 1 collagen is known to be able to bind relevant proteins (fibronectin and vitronectin), which affect the early adhesion of bone cells and their precursors cells (Geissler et al. 2000; Schliephake et al. 2005b).

Previous *in vivo* studies have suggested that the initial biological events occurring at the implant interface may also be tailored by the deposition of organic collagen matrix on titanium surfaces. In these studies, use was made of adsorption or biomimetic processes to deposit type 1 collagen on titanium implants (Schliephake et al. 2005a, 2009; Rammelt et al. 2006; Stadlinger et al. 2007, 2008a,c). In a canine model, Schliephake et al. (2005a) used screw-type implants with a collagen coating anchored on the surface by adsorption, which resulted in a significant increase in bone formation after 1 month. A positive effect of this type of coating was also shown in rat tibiae by Rammelt et al. (2006). Furthermore, a pig mandible was chosen to evaluate the suitability of ECM-based coatings as applied on square designed implants (Stadlinger et al. 2008a). The type 1 collagen coatings showed satisfactory rates of *de novo* bone formation especially within the first weeks to months. The same research group used circular implants with two defined recesses (Stadlinger et al. 2008b) and found that the biomimetic application of calf skin collagen coatings have an advantageous effect on peri-implant bone formation.

Although in the current study, the amount of newly formed bone was greater around the collagen-coated implants, no consistent significant favourable effect of collagen coatings on the bone response could be proven. Several explanations can be given for this discrepancy in observation compared with earlier *in vitro* as well as *in vivo* studies. First, the currently used implant design represents a clear challenge to bone formation at the implant surface as the occurrence of osteoconduction is almost excluded and new bone formation has to be evoked by the osteogenic properties of the implant surface. Secondly, the initial fixation of the used implants was more unfavourable in the dog mandible as explained above. Thirdly, the other groups that observed *in vivo* improvement of bone healing around implants applied a bovine collagen coating (Geissler et al. 2000; Schliephake et al. 2005a; Rammelt et al. 2006; Stadlinger et al. 2007). In this study, commercially available rat tail collagen type 1 was used (de Jonge et al. 2010). Presently, it cannot be excluded that this source of the collagen evokes an antigenic effect in a different species, like the dog.

Future studies have to pay attention to these issues to determine the final efficacy for organic ECM-based coatings to improve osseointegration of implant.

CaP nanoparticle coatings

Calcium phosphate coatings are known to enhance bone formation at the implant–bone interface. To overcome some drawbacks of commonly used coating techniques, ESD was introduced to allow the production of nanometre thin coatings with a standardized morphology as well as chemical composition (Leeuwenburgh et al. 2006; de Jonge et al. 2008). A recent *in vitro* study showed an increased adhesion of osteoblast-like cells to such coatings (de Jonge et al. 2009). This *in vitro* effect was confirmed in a rat study, which proved that an ESD-deposited nano-CaP coating significantly improved bone-to-implant contact compared with non-coated surfaces (Schouten et al. 2010).

Nevertheless, the ESD nano-CaP-coated implants did not increase the overall peri-implant bone formation in a significant manner in the current dog study compared with non-coated surfaces. Still, it has to be noticed that at 4 weeks after implantation, the nano-CaP-coated implants showed more bone deposition at the inner zone compared with the non-coated implants. The reason why no effect was seen at 12 weeks and why the nano-CaP coating was not able to evoke complete gap bridging after 12 weeks of implantation remains unclear. It is possible that the prolonged presence of a fibrous layer around the gap designed implants results in too early and faster dissolution of a nano-thin CaP coating (Siebers et al. 2007). In agreement, an *in vivo* study by Meirelles et al. (2008) was also unable to confirm the supportive effect of nano-CaP coatings on bone formation. In their experimental set-up, implants were placed in the rabbit tibia with a surgical gap of 0.35 mm on each implant side. Implant stability was warranted by a fixation plate and two additional screws.

On the other hand, an advantageous effect of discrete crystalline deposition of nano-CaP onto dual acid-etched implants was reported in various dog as well as human clinical trials (Granato et al. 2009; Vignoletti et al. 2009; Coelho et al. 2010; Telleman et al. 2010). However, Mendes et al. (2007, 2009) hypothesized on basis of their rat studies that the increase in the created complexity of the implant surface is probably more the reason for the bone-bonding mechanism than the calcium phosphate chemistry.

Conclusion

Within the limitations of the used experimental gap model, the obtained data did not provide a final answer on the possible favourable effect on bone formation of an ESD-deposited collagen coating on a titanium implant after 3 months of implantation in the dog mandible. Similarly, the data could not confirm the effect of a nanometre thin CaP coating to enhance bone healing into a gap-implant model. It can be hypothesized that the source of the collagen as well as the limited osseous environment overshadowed a possible effect of the applied implant surface modifications.

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