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Effects of implant geometry, surface properties, and TGF-β1 on peri-implant bone response: an experimental study in goats

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Key words: calcium phosphate coating, electrostatic spray deposition, goat study, implant design, TGF-β1

Abstract

Objectives: Despite the high success rates in implantology, the desire to use oral implants in more challenging clinical situations drives the need for continuing refinements in implant design and surface properties. In the present study, the effect of implant geometry on implant bone response was evaluated using two geometrically different implant types, i.e. screw type (St) and push-in type(Pi). Furthermore, the potential beneficial effect of an electrosprayed calcium phosphate (CaP) coating, either or not enriched with the osteoinductive growth factor TGF-β1, on the osteogenic response was examined.

Material and methods: A total of 54 implants, divided into six groups ($n = 9$), were inserted into the femoral condyles of nine goats. After an implantation period of 12 weeks, retrieved specimens were evaluated histologically and histomorphometrically. Measurements were statistically evaluated using SPSS 14.0 and analyzed using a linear regression model.

Results: With respect to implant design, St-implants showed an overall superior biological healing response compared with Pi-implants. Considering surface properties, the deposition of an electrosprayed CaP (2–3 μm) coating onto implants significantly increased the amount of bone–implant contact for both implant types. Additional enrichment of the CaP coating with the osteoinductive growth factor TGF-β1 did not significantly affect peri-implant bone response.

Conclusions: The results of this study indicate that a substantial improvement of the osteogenic response to titanium implants can be achieved by the deposition of an electrosprayed CaP coating. The enrichment of the coating with 1 μg TGF-β1 has only a marginal effect.

For decades, an uneventful healing period for several months has been advocated for oral implants to avoid fibrous tissue formation, which should result in an appropriate fixation of the implant within the native bone tissue (Adell et al. 1981). As during this healing period the implants should be prevented from any form of loading, patients experience this 'waiting period' as very inconvenient (Gapski et al. 2003). Therefore, despite the high clinical success rates of oral implants (Albrektsson et al. 1988; Buser et al. 1994; Testori et al. 2002; Del Fabbro et al. 2006), optimization of the initial bone-healing response after implant placement is still an important issue. In addition, the desire to use oral implants in more challenging clinical situations drives the need for continuing refinements in implant design, surface characteristics, and optimization of the biological healing response following implant placement (Davies 2003).

Over the last few decades, many different implant designs have been introduced

to optimize the anchorage of an implant in bone tissue, i.e. the rapid achievement and maintenance of direct bone–implant contact (Pilliar et al. 1991). Implant designs range from threaded to non-threaded, cylindrical or conical, either or not combined with additional features, such as vents, grooves, indentations, or perforations (Siegele & Soltesz 1989; Misch 1999; Sykaras et al. 2000; Steigenga et al. 2003). Despite the available data on the effect of single implant designs, the number of studies that directly compare different implant geometries is limited.

In addition to implant design, implant surface properties are an important factor in the biological healing response. Consequently, surface modifications represent a tool to enhance the biological response into a desired direction. The main consequences of the available surface modification techniques can be reduced to effects on surface topography (roughness or texture) or surface chemistry (or a combination thereof) (Puleo & Thomas 2006). It has been claimed that a certain degree of roughness leads to an increased mechanical stability through surface enlargement and mechanical interlocking with the surrounding bone (Shalabi et al. 2006; Duyck et al. 2007). In addition, some studies indicate that rougher surfaces increase the degree and rate of peri-implant bone formation (Puleo & Thomas 2006).

Regarding surface chemistry, beneficial effects on bone response have been ascribed to the deposition of ceramic materials, such as calcium phosphates (CaP) (Jansen et al. 1991; Dorozhkin & Epple 2002) and bioglass (Turunen et al. 1998; Wheeler et al. 2001). These so-called 'bioactive' materials have the ability to bond bone directly, without an intervening layer of soft tissue, thereby making them suitable as coatings onto bone-anchored implants (Ducheyne et al. 1992; Dorozhkin & Epple 2002). Various techniques have been used to deposit CaP coatings onto an implant surface, among which plasma spraying and magnetron sputtering are the most widely used. Although the osteoconductive and bone-bonding behavior of plasma-sprayed and magnetron sputter coatings has been confirmed by numerous studies (Jansen et al. 1993; Dhert 1994; Hulshoff & Jansen 1997; Lacefield 1998, 1999; Geesink 2002), there are still some important limitations related to these techniques. Plasma-sprayed coatings are relatively thick (>30 μm), which may result in coating delamination and fragmentation, thus influencing the long-term stability of the implants (Sun et al. 2001). Moreover, the crystallinity, composition, and thickness of the coatings are not uniform. Using the magnetron sputter technique, thinner (<5 μm), more dense and adherent coatings can be obtained (Jansen et al. 1993), but it is still challenging to vary the coating composition. These problems can be overcome using the Electrostatic Spray Deposition (ESD) technique, which is a simple and versatile technique that allows deposition of thin (2–3 μm) CaP coatings with a wide variety of chemical (Leeuwenburgh et al. 2004, 2005) and morphological characteristics (Leeuwenburgh et al. 2005, 2006).

Bone-healing responses can be further stimulated using appropriate growth factors (Fischer et al. 2003). Among these factors, members of the TGF-β superfamily appear to play the most critical role in bone healing (Gombotz et al. 1993). For example, TGF-β1 has been shown to stimulate bone healing in several animal models (Joyce et al. 1990; Beck et al. 1998). Moreover, previous studies have reported on the ability of TGF-β1 to enhance bone healing around ceramic-coated implants in unloaded gap-healing models (Sumner et al. 1995; Lind et al. 1996a, 1996b, 2001).

In view of the described effects of implant design, surface properties, and enrichment with biologically active factors, the present study aimed at evaluating these parameters in a comparative study design. Therefore, two geometrically different dental implants [screw type (St) and push-in type(Pi)] were used in an implantation study in goats. Additionally, these implants were modified using an electrosprayed CaP coating, either enriched or not with the osteoinductive growth factor TGF-β1. Implants were inserted into the trabecular bone at the medial side of both femoral condyles for 12 weeks. Evaluation consisted of qualitative (histology and fluorochrome labeling) as well as quantitative (histomorphometry) analyses of the peri-implant bone response.

Material and methods

Materials

Prototypes of two geometrically different oral implants, made of Ti-6AL-4V alloy, and with a smooth surface (Fig. 1) were provided by Dyna Dental Engineering BV (Bergen op Zoom, the Netherlands):

Fig. 1. Representation of implant designs used in this study: Screw type (St; left) and Push-in (Pi; right).

- Cylindrical, St-implant (diameter: 4.2 mm; length: 10 mm)
- Cylindrical, Pi-implant with grooves (diameter: 3.6 mm; length: 10 mm)

Recombinant human TGF-β1 was obtained from R&D Systems Inc. (Minneapolis, MN, USA). Fluorochromes Alizarin (Alizarin Complexon dehydrate), Calcein (Fluorexon), and Tetracycline were acquired from Acros Organics (Geel, Belgium).

Electrostatic spray deposition (ESD) process

Before CaP coating deposition, implants were cleansed ultrasonically in nitric acid 10% (15 min), acetone (15 min), and ethanol (15 min) successively. For CaP coating deposition, a vertical ESD setup (Advanced Surface Technology, Bleiswijk, the Netherlands) was used, as described previously by Leeuwenburgh et al. (2003). In brief, the basic principle of ESD is the generation of a so-called electrospray of organic solutions containing the inorganic precursors Ca and P. This is accomplished by pumping this solvent through a nozzle, which is connected to a high-voltage supply. As a result of the applied potential difference, a spray consisting of charged, micron-sized droplets is formed, which are attracted towards a grounded and heated substrate. Consequently, the droplets impinge onto the heated substrate, where they lose their charge. After complete solvent evaporation, a thin layer (2–3 μm) consisting of

the inorganic product is left on the substrate surface.

In this study, implants were coated in three runs (in turns of 120°) of 45 min each, at a substrate holder temperature of 475°C, a nozzle-to-substrate distance of 20 mm, and a precursor liquid flow rate of 2.0 ml/h. Coatings were prepared using precursor solutions with a Ca/P ratio of 1.8 ($Ca(NO_3)_2 \cdot 4H_2O$ and H_3PO_4 were precursors for Ca and P, respectively). Subsequently, all coated implants were subjected to an additional heat treatment for 2 h at 700°C in order to transform the amorphous coatings into crystalline carbonated hydroxyapatite (CHA) coatings (Siebers et al. 2007). The morphology of the CaP coatings was characterized using scanning electron microscopy (SEM, Jeol, SEM6310, Tokyo, Japan). In addition, X-ray diffraction (XRD) using a thin-film Philips X-ray diffractometer (PW3710, Almelo, the Netherlands) and Fourier Transform Infrared Spectrometry (FTIR; Perkin Elmer Instruments, Zoetermeer, the Netherlands) were used in order to characterize the crystal structure and the molecular structure of the deposited coatings, respectively.

TGF-β1 loading

For growth factor loading, TGF-β1 was dissolved in sterile 4 mM HCl containing 1 mg/ml bovine serum albumin (BSA; Sigma Aldrich, Zwijndrecht, the Netherlands). Administration was achieved by direct adsorption of the growth factor onto the CaP-coated implants. Aseptic conditions were maintained throughout the adsorption process. A volume of 6 µl of the HCl/BSA solution (containing 1.0 µg TGF-β1) was pipetted onto each implant. After loading, implants were immediately stored at −80°C for 1 h, and placed in a 24-well plate for overnight lyophilization.

Experimental animal groups

In the animal study, a total of six different experimental groups were used:

1. St (Screw type), non-coated;
2. St + CaP (Screw type with a CaP coating);
3. St + CaP + TGF-β1 (Screw type with a CaP coating loaded with TGF-β1);
4. Pi (Push-in type), non-coated;
5. Pi + CaP (Push-in type with a CaP coating); and
6. Pi + CaP + TGF-β1 (Push-in type with a CaP coating loaded with TGF-β1).

Sterility was obtained through autoclavation of non-coated and CaP-coated implants. TGF-β1 enrichment of CaP-coated implants was performed after autoclavation under aseptic conditions.

Surgical procedure

All *in vivo* work was conducted in accordance with ISO standards, and protocols of the University Medical Center, Nijmegen, the Netherlands. National guidelines for the care and use of laboratory animals were observed, and approval of the Experimental Animal Ethical Committee was obtained. A total of 54 implants (nine implants of each experimental group; $n = 9$) were implanted into nine female Saanen goats (2–4 years of age), with a mean body weight of about 50–60 kg. Surgery was performed under general inhalation anesthesia and sterile conditions. To reduce the perioperative infection risk, the prophylactic antibiotic Albipen® was administered subcutaneously (Albipen 15%, 3 ml/50 kg preoperative and Albipen LA, 7.5 ml/50 kg for 3 days post-operative, Intervet BV, Boxmeer, the Netherlands). Anesthesia was initiated by an intravenous injection of Pentobarbital® (AUV Wholesale, Cuijk, the Netherlands). Subsequently, the goats were intubated and connected to an inhalation ventilator with a constant volume of a mixture of nitrous oxide, isoflurane, and oxygen. Before the insertion of the implants, each animal was immobilized on its back and the hind limbs were shaved, washed, and disinfected with povidone–iodine. For implantation of the implant in the femur, a longitudinal incision was made on the medial surface of the left and the right femur. Subsequently, landmarks were placed in the femoral condyle, and a radiographic image was made to localize the trabecular bone. After exposure of the femoral condyle, a 2 mm pilot hole was drilled, which was gradually widened with drills of increasing size until the final diameter was reached. The final drill, For both the St and the Pi implants had a diameter of 4 mm. The bone defect preparation was performed with a gentle surgical technique, using low rotational drill speeds (800–1200 rpm) and continuous external cooling with saline. In this way, three holes were made on the medial side of the condyle (proximal, medial, and distal with an interimplant distance of 1 cm). After preparation, the holes were irrigated and the implants were inserted. In each femur, three implant sites were located, resulting in six sites per goat (Fig. 2). To ensure complete randomization, the implants were placed according to a balanced split-plot design. However, to avoid cross-over effects, all implants containing TGF-β1 were grouped together and placed in left femurs only. After insertion of the implants, the soft tissues were closed in separate layers, and the skin was closed transcutaneously using resorbable Vicryl® 4·0 sutures (Ethicon Products, Amersfoort, the Netherlands). To reduce pain after surgery, all goats received Finadyne® (AUV Wholesale, Cuijk, the Netherlands) for 2 days postoperatively. Postoperative radiographs were conducted to check implant placement. To visualize the dynamics of bone growth, four goats received sequential fluorochrome labels at 1, 6, 9, and 11 weeks postoperatively (Table 1). All labels were administered subcutaneously at 25 mg/kg body weight. At 3 months postimplantation, euthanasia was performed with an overdose of

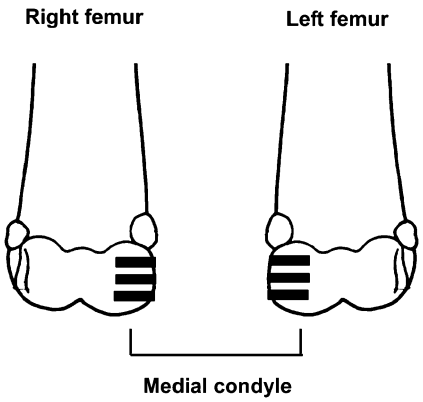

Fig. 2. Location of implant sites in the medial femoral condyle. The black bars represent the implants.

***Table 1*. Scheme for subcutaneous fluorochrome administration at timed intervals**

Weeks before sacrifice	Fluorochrome marker	Color	Dose (mg/kg)
11	Tetracycline	Yellow	25
9	Calcein	Green	25
6	Alizarin complexon	Red	25
1	Calcein blue	Blue	25

Nembutal®, and the implants with surrounding tissue were retrieved for histological evaluation.

Histological preparations

After the animals were sacrificed, the femoral condyles were retrieved, excess tissue was removed, and, using a diamond circular saw, the condyles were divided into smaller specimens suitable for histological processing. Finally, each specimen contained only one implant with surrounding bone. Subsequently, the tissue blocks were fixed in 10% neutral-buffered formalin solution, dehydrated in a graded series of ethanol (70–100%), washed with acetone, and embedded in methyl methacrylate for 4 weeks. After polymerization, non-decalcified thin longitudinal sections (10–20 μm) of the implants 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. Specimens from the four goats that received fluorochromes were processed to obtain both unstained and stained sections.

Histological and histomorphometrical evaluation

To evaluate the trabecular bone response to the implants, histological as well as histomorphometrical analyses were performed. Histological evaluation using a light microscope DMRD (Leica Microsystems AG, Wetzlar, Germany) consisted of a concise description of the observed tissues reaction, including the structure and arrangement of cells, implant, and tissue–implant interface. In addition, a computer-based image analysis technique (LEICA QWIN PRO-IMAGE analysis software; Leica Imaging Systems, Cambridge, UK) was used for histomorphometrical evaluation. The quantitative measurement was performed for three different sections per implant, on each side of the 2D histological image. The average of these six measurements was used for statistical analysis. The quantitative parameter assessed was the bone–implant contact (at magnification × 25). Therefore, the amount of bone contact was defined as the percentage of implant length at which there is direct bone-to-implant contact without intervening soft tissue layers. Measurements were performed along the length of the implant: for the St-implant, starting at the first coronal screw thread, and for the Pi-implant, starting at the beginning of the first concave thread (total standardized distance of interest of 4000 μm).

Fluorochrome labeling

For fluorochrome labeling analysis, a reflectant fluorescence microscope was used (Leica Microsystems AG). In addition, a Zeiss filter No. 05, consisting of a 395–440 nm band-pass excitation filter, was used for visualization of the different fluorochromes.

Statistical analysis

Measurements were statistically evaluated using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Data obtained with histomorphometry regarding bone–implant contact were analyzed using a linear regression model. The dependent variable was represented by 'bone contact,' whereas the independent variables were represented by 'implant design', 'presence of CaP,' and 'presence of TGF-β1.' Using this model, the effect of the independent variables on bone–implant contact could be evaluated. The significance level was set at a probability (P) value smaller than 0.05.

Results

Coating characterization

SEM observations showed a uniform surface coverage of the implant. The electrosprayed CaP coatings revealed a porous, reticular surface morphology (Fig. 3). Heat treatment increased coating crystallinity and yielded in a CHA structure as confirmed with XRD and FTIR (data not shown). Additionally, XRD measurements showed reflection peaks of TiO_2 at 27.6 and 36.2° 2θ for heat-treated CaP coatings, which were absent in non-heat-treated CaP coatings. The coatings were crack free after the heat-treatment at 700°C.

General observations of experimental animals

All nine goats remained in good health during the experimental period without any postoperative wound-healing complications. At sacrifice, no signs of inflammation or adverse tissue reaction could be seen around the implants. Table 2 depicts the number of implants placed, retrieved after implantation, and included in the histological and histomorphometrical analyses. Of the 54 installed implants, a total of 53 implants could be retrieved. One implant was not found during retrieval. Throughout the histological processing, five implants were damaged, and hence not included for analyses. A total of 48 implants were used for analyses.

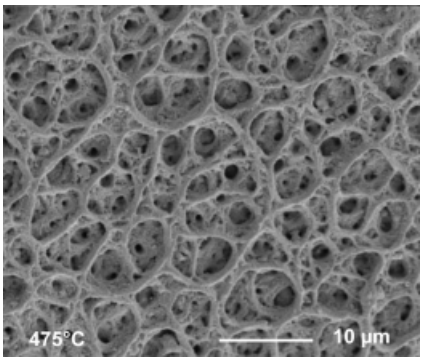

Fig. 3. Scanning electron micrograph of the porous CaP coating as deposited using electrostatic spray deposition (ESD).

***Table 2*. Number of implants placed, retrieved and used for histological and histomorphometrical analyses**

	# implants placed	# implants retrieved	# implants used for analyses
St			
Non-coated	10*	10	9‡
CaP	9	9	9
CaP + TGF-β1	8*	8	7‡
Pi			
Non-coated	9	8†	8
CaP	9	9	8‡
CaP + TGF-β1	9	9	7‡

*During implantation one St + CaP + TGF-β1 implant was lost, and replaced by one non-coated St-implant.
†During explantation one non-coated Pi-implant could not be retrieved.
‡During histological preparation one non-coated St-implant, one St + CaP + TGF-β1, one Pi + CaP, and one Pi + CaP + TGF-β1 implant were damaged.

Descriptive histological evaluation

Light microscopic examination of the methylene blue/basic fuchsin-stained sections of the implants and its surrounding tissue demonstrated that one of the implants (non-coated Pi) was inserted very close to the growth plate cartilage, but no contact between the implant and

the growth plate was seen. Also, for another implant (St + CaP + TGF-β1), inflammatory cells were observed at the implant surface. Generally, in all sections, bone apposition, bone remodeling, and ingrowth of newly formed bone into the threads of the implants were observed.

In all St-implants, except for one, a close contact between the implant and surrounding bone was observed. The screw threads were almost completely filled with bone (Fig. 4, St-implants), but around one non-coated St-implant, an intervening fibrous tissue layer was present at the crestal area of the implant. In the sections of four Pi-implants (three non-coated, and one CaP coated), a gap was observed between the surrounding bone and the implant surface (Fig. 4, Pi-implants), resulting in no implant–bone contact at all. In all other Pi-implants a close bone-to-implant contact was observed.

For all St- and Pi-implants, except for one, the CaP-coated equivalents, whether or not enriched with TGF-β1, showed a close bone-to-implant contact (Fig. 4, CaP coated implants). In these sections, the presence of bone on top of the thread and conducting over the implant surface into the threads was observed.

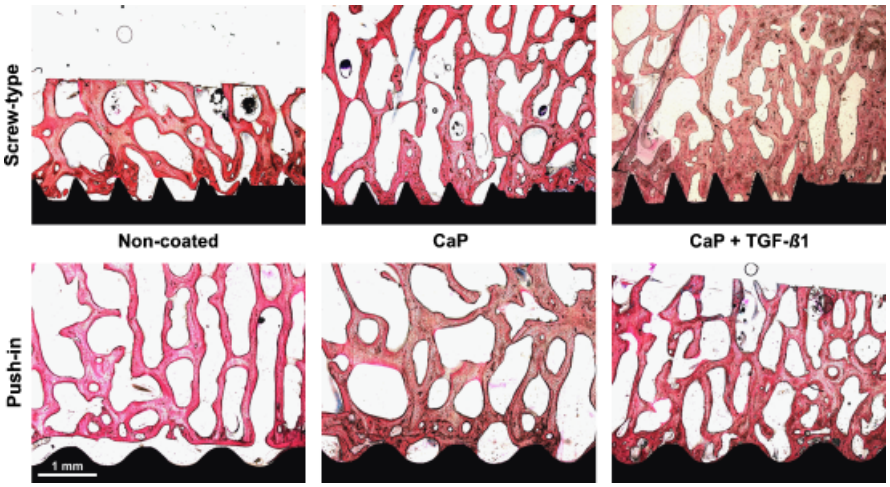

Fig. 4. Representative histological sections of Screw-type (St) and Push-in (Pi) implants after 12 weeks of implantation in the femoral condyles of goats.

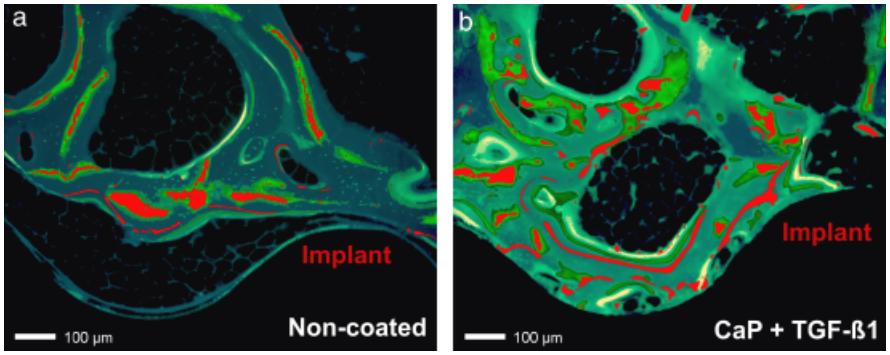

Fig. 5. Fluorescence microscopy images of histological sections of fluorochrome-administered experimental animals. Colors indicate bone formation at 1 (blue; calcein blue), 6 (red; alizarin-complexon), 9 (green; calcein green), and 11 (yellow; tetracycline) weeks post-implantation.

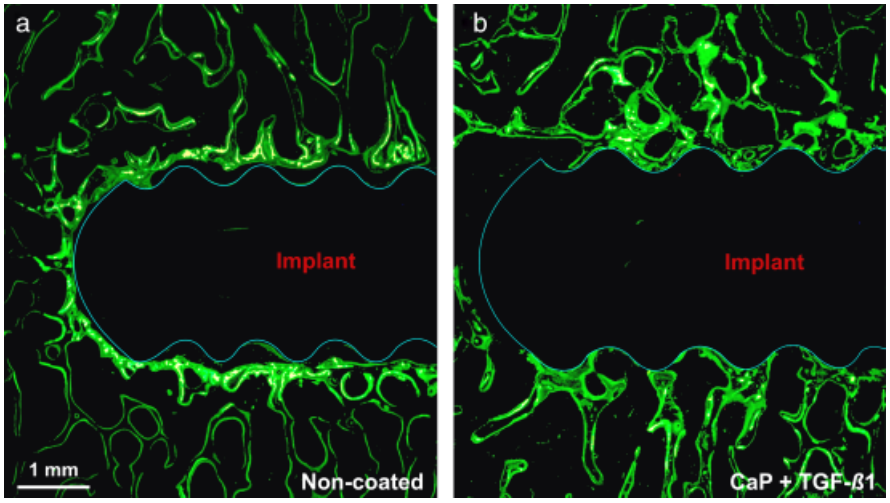

Fig. 6. Fluorescence microscopy images of histological sections of fluorochrome-administered experimental animals. The color indicates bone formation at 9 weeks post-implantation (green; calcein green). Images indicate bone formation predominantly in the implant vicinity.

Fluorescence microscopy

Consecutive fluorochrome markers, laid down in the form of bands, were observed around the implants in the sequence of their administration (Fig. 5a and b). In contrast to the labels, alizarin complexon (red), calcein (green), and tetracycline (yellow), the presence of the calcein blue label, administered 1 week after implantation, could not be clearly identified.

Detailed observation of the images showed the yellow line to be very close to the implant surface and bone marrow spaces in the trabecular bone, and the green and red lines more towards the peripheral areas (away from the implant surface) with about equal distances between the lines. This band formation was absent in images of the non-coated groups.

Fluorochromes in the non-coated groups of both implant types, i.e. St and Pi, showed a less intense fluorescence signal compared with the surface-modified groups, i.e. CaP coated, enriched with TGF-β1 or not (Fig. 6a and b). In general, a more pronounced signal was seen at sites where new bone was formed.

Histomorphometrical analysis

The results of the bone–implant contact measurements and the outcome of the

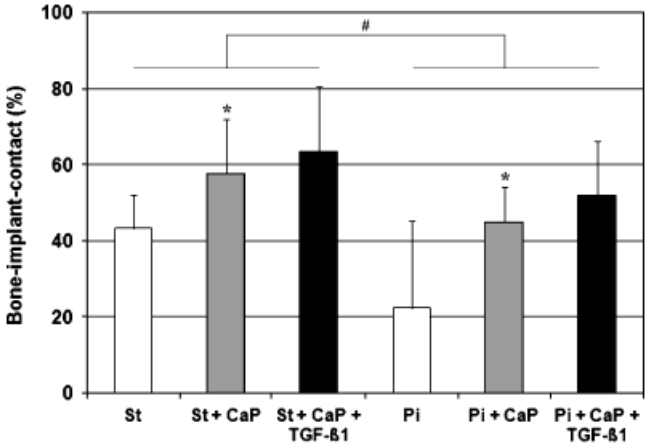

Fig. 7. Results of histomorphometric analyses of Screw-type (St) and Push-in (Pi) implants in femoral condyles of goats. Bone-implant contact of St- and Pi-implants after 12 weeks of implantation is displayed for the different experimental groups. Bars represent the mean + standard deviation. No interaction between the experimental variables was found. Significantly higher bone implant contact for St-implants compared with Pi-implants. *Significantly different compared with non-coated controls.

***Table 3.* Bone contact percentages and statistical testing of the experimental variables**

Bone-implant contact	95% Confidence Interval			
	Effect	Lower	Upper	*P*-value
Intercept	60.1	44.2	76.1	<0.1%
Implant design (Pi = 1, St = 0)	− 12.5	− 20.9	− 4.2	0.4%*
CaP (with = 1, without = 0)	19.7	10.4	28.9	<0.1%*
TGF-β1 (with = 1, without = 0)	8.0	− 2.9	18.9	14.7%

*Indicates statistically significant effect ($P<0.05$).
CaP, calcium phosphate; TGF-β1, transforming growth factor-β1.

statistical analyses are depicted in Fig. 7 and Table 3, respectively. Considering geometry, a significantly higher (12.5%) bone–implant contact was observed for St-implants compared with Pi-implants ($P=0.004$). Furthermore, surface modification using the electrosprayed CaP coating showed a significantly higher (19.7%) bone contact ($P<0.001$) compared with their non-coated equivalents. The enrichment of CaP-coated implants with TGF-β1, however, was not statistically significant (8.0%; $P=0.147$) compared with CaP-coated implants.

Discussion

The aim of this study was to evaluate the effect of implant geometry on bone-healing responses in an *in vivo* goat femoral condyle model. Furthermore, the potential beneficial effect of an electrosprayed CaP coating, enriched or not with TGF-β1, on bone healing was evaluated. The results of this study showed a significant effect on the bone-healing response of both implant geometry and CaP coating, whereas additional enrichment of CaP-coated implants with TGF-β1 did not further enhance peri-implant bone response.

A prerequisite for successful orthopedic and dental implant placement is to obtain a good primary stability of the implant in the surrounding bone (Albrektsson et al. 1981). The establishment of such a mechanically stable interface prevents the development of an intervening layer of fibrous tissue (Shalabi et al. 2006). In view of the currently available implant systems, it needs to be emphasized that the degree of primary stability, besides bone quality, largely depends on implant design and insertion modality. For this reason, the current study included two geometrically different implant designs (St; Pi). With respect to implant geometry, the results of this study indicate that bone–implant contact is superior for St- over Pi-implants. This observation corroborates published data of other experiments, in which different animal models were used (Steigenga et al. 2003; Watzak et al. 2005). An explanation for the higher implant–bone contact for St-implants is that, relative to their length, St-implants offer the surrounding bone a larger surface area compared with Pi-implants. Moreover, the manufacturer's recommendations for insertion of the St-implants actually involve screwing implants into an undersized defect, whereas the advice for Pi-implants is to push the implants into an oversized defect. Consequently, the placement of St-implants is associated with shear forces at the interface, hence loosening small bone fragments. These bone fragments are likely to be pressed in between the trabecular voids and in between the screw threads during implant placement, enhancing new bone formation (Shalabi et al. 2006). In addition, during implant placement, a degree of compression will take place along the implant–bone interface, which further enhances implant stability (O'Sullivan et al. 2004). When such an undersized approach is used for cylindrical Pi-implants, there may be an increased risk of (i) failing to place the implant fully into the drill hole and (ii) detrimental effects of bone tissue compression, potentially causing local cellular damage, resulting in cell death, necrosis, and ultimately bone resorption (O'Sullivan et al. 2004). Because of the visco-elastic properties of bone, an oversized bone cavity is always drilled for cylindrical implants, implicating less primary stability as compared with St-implants.

To improve bone–implant contact, bioactive materials, such as CaP ceramics, have been used successfully. These materials are characterized by their potential to form a very tight, chemical bond with the surrounding bone, i.e. 'bone–bonding' (Ducheyne et al. 1992). Because of their favorable biological behavior, bulk CaP ceramics have been widely used in the orthopedic and dental field. As bulk materials, however, the mechanical properties of CaP ceramics are too weak, limiting their use in load-bearing situations. Consequently, CaPs are applied as coatings onto mechanically strong (metallic) implants. Numerous studies have already confirmed the osteoconductive and bone–bonding behavior of CaP coatings (Jansen et al. 1991; Hulshoff & Jansen 1997). Similar positive data were obtained in an

animal gap study, showing that gaps of 1 mm can only be bridged by bone if a CaP coating is applied, whereas uncoated implants demonstrated no bone contact at all (Clemens et al. 1997).

Various deposition methods have been proposed to apply CaP coatings onto implants, among which plasma spraying and magnetron sputtering are the most widely used. A major drawback of both coating techniques is the lack of control over the final coating composition and morphology. Consequently, promising CaP phases, like carbonate apatite, which comprise a chemical composition closest to bone and teeth, cannot be deposited (Jansen et al. 1993; De Groot et al. 1994; Leeuwenburgh et al. 2004). To overcome these problems, the ESD technique was used in the present study to deposit carbonated apatitic CaP coatings. Leeuwenburgh et al. (2003) already confirmed the feasibility of the ESD technique for the deposition of thin CaP coatings with a defined surface morphology, by varying the deposition parameters. Moreover, by varying the Ca/P precursor solution ratio, and applying an additional heat treatment, this technique is able to control the coating composition and crystallinity (Leeuwenburgh et al. 2004). Despite the improved mechanical strength and interfacial adhesion, no data are available yet that can provide information regarding whether the coating remains stable after implantation. However, the observed biological response in the present study proves that during the initial bone-healing process, the CaP coating exerted its biological effect by enhancing the bone formation around the implanted materials. Additionally, as a result of the heat-treatment, oxidation of the titanium substrate occurred, which was demonstrated through XRD analysis. Although the authors are aware of the effects of heat-treatment on the mechanical properties of titanium, the current study did not evaluate this parameter.

In addition to the deposition of a CaP coating, the potential beneficial effect of TGF-β1 on the peri-implant bone response was also evaluated in this study. It is recognized that TGF-β1 might have effects in the vicinity of the implant; however, thorough histological observations did not support this suggestion. With respect to the study design, the combination of TGF-β1 with a non-coated implant was not included. It should be realized that most growth factors, including TGF-β1, are very expensive. Only a significant extra effect, beyond the effect of a CaP coating alone, may value these high costs. To prove such an effect, only the combination of CaP-coated implants and TGF-β1 was selected. Over the last decade, several studies have been performed to evaluate the effects of TGF-β1 *in vitro* and *in vivo*, which obtained rather mixed results. For example, 2 μg TGF-β1 applied in a 3% methylcellulose gel was able to regenerate a critical-size defect in a rabbit skull within 28 days; 0.1- and 0.4 μg showed less bone formation (Beck et al. 1991). In a non-critical-size rabbit skull model, the combination of a Ti fiber mesh implant with 2 μg TGF-β1 induced orthotopic bone formation (Vehof et al. 2002). Further, in a canine model, porous coated implants showed a more effective bone ingrowth with a dose of 120 μg TGF-β1, compared with a dose of 335 μg TGF-β1 (Sumner et al. 1995). In the present study, no significant effects of TGF-β1 on bone healing were observed. The reason for this lack of beneficial effects of TGF-β1 remains unclear. A possible explanation may be the dose–response effect for TGF-β1. However, as shown in the available literature, higher doses do not necessarily generate more bone formation, as there is an optimum dose. Therefore, in the present study, it was decided to load the implants with 1 μg TGF-β1. In addition to the dose-response effect, data have been published suggesting a positive correlation between the *in vivo* osteoinductive activity of TGF-β1 and the amount of protein retained at the site of implantation. Consequently, if less growth factor is retained, a higher dose is needed for the same osteoinductive response. Although growth factor retainment was not assessed in this study, the amount of TGF-β1 remaining on the implants in the present study might have been below the optimal osteoinductive level. Several *in vitro* studies have been performed to determine the release characteristics of TGF-β1. For instance, a burst release of 70% of TGF-β1 was observed from a titanium fiber mesh implant (Vehof et al. 2002). Lind et al. (1996b) found a burst release of 80% of TGF-β1 adsorbed onto tricalcium phosphate-coated implants. Loading an electrosprayed β-tricalcium phosphate coating with TGF-β1 also resulted in a burst release of >90% (Siebers et al. 2006). Analogous to this relatively fast and almost complete *in vitro* release of TGF-β1, the release in the current study may have resulted in suboptimal amounts of TGF-β1 both spatially and temporarily. Therefore, the need for a slow and more gradual release still exists and has to be investigated. A possible solution can be found by the incorporation of the growth factor into the carrier material, as proposed by Liu et al. (2004), who incorporated BMP-2 during the deposition of a biomimetic CaP coating onto a titanium implant by co-precipitation. More research is needed to find a suitable technique for the incorporation of biologically active compounds in order to create a more gradual release, or an optimized combination of a burst and sustained release profile.

In summary, this study demonstrates that the peri-implant healing response following implant placement is dependent on both implant design and surface modification. With respect to implant design, St-implants show an overall better biological healing response over Pi implants. Considering surface modification, the deposition of an electrosprayed CaP coating onto implants significantly increased the amount of bone–implant contact. Further, enrichment of the CaP coating with the osteoinductive growth factor TGF-β1 did not show an additional effect on peri-implant bone response. The results of this study consequently indicate that a substantial improvement of the osteogenic response to titanium implants can be obtained by the deposition of an electrosprayed CaP coating. The enrichment of the coating with 1 μg TGF-β1 has only a marginal effect.

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