CLINICAL ORAL IMPLANTS RESEARCH

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A novel implantation model for evaluation of bone healing response to dental implants: the goat iliac crest

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Abstract

Objectives: Despite the availability of numerous animal models for testing the biological performance of dental and orthopedic implants, the selection of a suitable model is complex. This paper presents a new model for objective and standardized evaluation of bone responses to implants using the iliac crest in goats.

Material and methods: The feasibility of the iliac crest model regarding anatomy and implant positioning was determined using two cadaveric specimens and the bone structure was evaluated and compared with that of the goat femoral condyle. Additionally, the validity of the model was tested by performing an *in vivo* study.

Results: By means of a rather simple, safe, fast and reproducible surgical procedure, the iliac crest in goats could be approached and allowed the implantation of maximally five dental implants per iliac crest. Because of the bilateral implantation possibility, statistical comparisons between groups on either side of the goat could be performed, resulting in a high statistical power, and hence a reduction in the number of animals required to obtain significant data.

Conclusions: In terms of surgical approach, anatomy and implant positioning, the iliac crest is the preferred model over the femoral condyle model. The iliac crest implantation model is suitable for evaluation of the osteogenic response to bone implant materials and represents a justified and deliberate alternative to the already existing animal models.

Over the last 40 years, the use of dental and orthopedic implants has become increasingly widespread, and still continue to expand. For successful clinical employment, implants should be designed in such a way that a controlled, guided and rapid healing at the bone–implant interface is induced upon implantation, resulting in stable anchorage of the implant in bone. Although excellent clinical results have already been claimed for a number of implant designs and surfaces, the optimization of implant characteristics remains a continuous process. Furthermore, the production and marketing of new brands persist and evolve at such a high pace that new companies enter the implant market without extensive preclinical and clinical testing (Albrektsson et al. 2008). In some cases, this attitude has resulted in the marketing of unsuitable implants and unnecessary failures (Albrektsson et al. 2008). To avoid such pitfalls, in-depth research still needs to be carried out before marketing of new implants, in order to obtain fundamental scientific information and data on the biological performance regarding a new or optimized product.

In order to determine whether a newly developed implant matches the requirements of compatibility to the biological surroundings, mechanical stability and,

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above all, safety, it must undergo thorough testing before clinical use. The first step that can provide useful information is *in vitro* testing. *In vitro* testing is frequently used to detect potential toxic and carcinogenic effects of a new material (Pearce et al. 2007). However, no *in vitro* cell culture system is able to mimic the tissue response. Therefore, the use of animal models is inevitable. Animal studies are also useful to test the histocompatibility and functionality of a specific implant, as beside tissue response, animal models allow the evaluation of implants in loaded or unloaded conditions. In clinical situations, dental and orthopedic implants are subjected to functional loading. If the loading aspects need to be evaluated, an animal model is required that reflects well the complex environment in clinical applications, in order to evaluate the osteogenic response at the bone–implant interface under the influence of loading (e.g. intra-oral implantation models (Deporter et al. 1986; Sagara et al. 1993; Fugl et al. 2009). In situations, in which the goal is solely to evaluate the biological response to the implant, a non-loading animal model is sufficient (e.g. intramedullary model; Feighan et al. 1995; Schouten et al. 2009b). It should be emphasized, however, that despite the fact that the biological conditions in animals more closely resemble the mechanical and physiological situation in humans, these will never be able to fully represent the clinical situation.

Currently, numerous animal models are available for testing the biological performance of newly developed implants. However, as the range of existing animal models is rather wide, the selection of an adequate and suitable model is complex. When choosing an animal model, one should realize that the healing response within different animals and tissues can vary significantly, implying that a thorough knowledge of the biological and physiological characteristics of the species is essential. Consequently, care must be taken regarding the selection of an appropriate animal model and implantation site to obtain reliable data (An & Friedman 1998a). The selection of a suitable implantation model largely depends on whether its anatomical and physiological characteristics meet the research demands. For example, when placing implants into the rabbit tibial or femoral bone, the implant dimensions are limited to a diameter of about 3.75 mm and a length of 8 mm, in view of the risk of inducing a pathological fracture (Meirelles et al. 2008; Susin et al. 2008; He et al. 2009). Consequently, evaluation of the biological performance of dental implants, having diameters ranging from 2.8 to 6 mm and a length between 7 and 15 mm is generally performed in larger animals, such as dogs, sheep and goats (Berglundh et al. 1994; Grizon et al. 2002; Schouten et al. 2009a). With respect to physiological characteristics, it is known that the metabolic and bone healing properties in rats as well as in rabbits are significantly different from those in the human bone, which makes the extrapolation of the results obtained difficult (An & Friedman 1998a; Pearce et al. 2007). Furthermore, for an accurate judgment of the biological behavior of implants, the animal experiments should be designed correctly and statistical analysis should be comparative (Jansen et al. 1996). In an ideal study design, all test variables are positioned in one animal, thereby making comparisons within one animal feasible, as such, deleting the effect of the animal itself. Moreover, the costs to acquire and care for the animals are important factors that need to be considered when deciding on the species of animal and a particular model. Although no single animal model will fully meet the requirements, there is always need for refinements in order to approximate the perfect model as much as possible. Finally, authorities on animal welfare are also becoming increasingly critical. Performing animal experiments requires approval of a committee dedicated to animal welfare and ethical issues related to this. These authorities urge the scientific community to improve the quality of the experimental design and to search for alternatives where possible according to their ground rules: replacement, reduction and refinement (three R's). This implies that researchers should (i) look for alternative techniques, such as *in vitro* cell culture models, cadaveric specimens or computer simulations that can 'replace' animals or at least use animals that are phylogenetically lower, (ii) 'reduce' the number of animals required and (iii) 'refine' the experimental method used by reducing the ethical costs in terms of painful or stressful procedures (An & Friedman 1998b). In view of the aforementioned, a new model for objective standardized evaluation of the bone response to implants is presented using the iliac crest in goats. This implantation model is supposed to conform to the requirements of (i) anatomical and physiological similarity to humans, (ii) balancing of experimental influences (implantation sites), (iii) appropriate size for the number and size of implants chosen and (iv) cost effectiveness. To verify the assumptions made on the iliac crest model, its feasibility was determined regarding anatomy and implant positioning using two cadaveric specimens, and the bone structure was evaluated and compared with that of the goat femoral condyle. Additionally, the validity of the model was tested by performing an *in vivo* goat study. This paper will present the iliac crest implantation model as an alternative to other models.

Currently available animal models

To determine to what extent the iliac crest implantation model is a useful and potentially more appropriate or justified alternative for the already existing animal models, the following section will summarize the most commonly used models for bone–implant interactions, which are: (i) tibia and (ii) femoral condyle.

Tibia

The tibia has been extensively used as the location for implant placement in rats, guinea pigs, rabbits, minipigs, dogs, goat and sheep. With respect to the surgical approach, the skin incision made to approach the tibial bone, can be located on either the medial or the lateral side of the tibia. Generally, implants can be placed bilaterally in both tibiae, allowing each animal to serve as its own control. Regarding bone remodeling rates, extrapolation of results obtained in rats as well as in guinea pigs, is limited (Pearce et al. 2007). With increasing animal size, implant size also increases: for rats: 1–1.9 mm (diameter), 2–2.5 mm (length) (Kajiwara et al. 2005; Wermelin et al. 2008), guinea pigs: 1.8 and 2 mm (diameter), 5–7 mm (length) (De Smet et al. 2006, 2008), rabbits: 3–3.75 mm (diameter), 8 mm (length)

(Meirelles et al. 2008; Susin et al. 2008; He et al. 2009), for minipigs: 4.1 mm (diameter), 10 mm (length) (Buchter et al. 2005a, 2005b), for dogs: 4–6 (diameter), 6–18 mm (length) (Cook et al. 1992; Coelho & Lemons 2009; Suzuki et al. 2009), for goats: 3.8–4.6 mm (diameter), 10–13 mm (length) (Vercaigne et al. 1998b; Schierano et al. 2005; Shalabi et al. 2007), for sheep: 6–6.5 mm (diameter), 15–18 (length) (Svehla et al. 2000, 2002). Besides variations in implant dimensions, the number of available implantation sites also differs significantly between animal species. In rats and guinea pigs, for example, only one implant site per limb has been reported. In rabbits, two implants can be placed (Susin et al. 2008). Minipigs, dogs and goats offer four implant sites, whereas in sheep, even five implant sites are available (Svehla et al. 2000, 2002). A summary of the aforementioned data with respect to implant dimensions and available implant sites per animal are given in Table 1.

Femoral condyle

Another frequently used implantation site for implant evaluation is the femoral condyle. A variety of implants can be placed in the femoral condyle, such as rods (Heimann et al. 2004; Stadelmann et al. 2008; Schouten et al. 2009c), plugs (Lind et al. 1996b; Clemens et al. 1998) and also screws (Caulier et al. 1995; Lind et al. 1996a; Hulshoff et al. 1997; Klokkevold et al. 2001; Schouten et al. 2009a; Susin et al. 2008; Nikolidakis et al. 2009), are commonly implanted in the femoral condyle of rats, rabbits, dogs, goats and sheep. Both surgical approach and exact implantation site can vary between experiments. The most common approach involves a longitudinal incision over the medial or lateral surface of the femoral condyle, allowing placement of the implant in a perpendicular direction to the long axis of the femur (Lind et al. 1996b; Peter et al. 2006; Nikolidakis et al. 2009). Alternatively, one can start with a medial parapatellar incision, followed by lateral dislocation of the patella and maximal flexion of the knee joint to allow implantation at the bottom of the femoral condyle (i.e. the area that is in almost constant contact with the tibial plateau (Schouten et al. 2009c)). The first approach, i.e. the medial or lateral approach, offers space for more than one implant per condyle, though the maximum number of implants to be placed differs between animal species. In rats, only one implant can be placed per condyle (Peter et al. 2006; Schouten et al. 2009c). The placement of two implants per site is possible in rabbits (Klokkevold et al. 2001), and sheep (Stadelmann et al. 2008), whereas in goats as much as three implants per condyle can be placed (Schouten et al. 2009a), resulting in a maximum of six implants per animal. For the second approach, i.e. the parapatellar approach, only one implant per condyle can be placed, irrespective of the animal species used. Except for number of implants per site, the diameter (dm) and length (l) of the implant also matter, i.e. 1.75–3 mm (dm) and 3.5–5 mm (l) in rats, 3.25–3.75 (dm) and 4–7 mm (l) in rabbits, 6 mm (dm) and 9–16 mm (l) in dogs, 3.6–9 mm (dm) and 8–10 mm (l) in goats and 3–13 mm (dm) and 5–130 mm (l) in sheep (Table 1). Regarding the femora in animals, implant placement is not only limited to the condyles, but studies have also been reported on using a transcortical approach, which allows for example in dogs, the placement of five implants per femur of 6 mm in diameter and 18 mm in length (Thomas & Cook 1985; Cook et al. 1992).

A novel implantation model to test the bone–implant interface: the goat iliac crest

In clinical practice, harvesting iliac bone grafts for the reconstruction of bone defects is a common procedure. Inspired by this, Anderson et al. (1999), validated the iliac wing as a suitable bicortical critical size defect model for the evaluation of bone response to biodegradable bone substitutes with a diameter of 17 mm. Since then, only two additional studies, one in goats (Kruyt et al. 2004) and one in rabbits (Ge et al. 2009) were published, using the same critical-sized iliac crest defect model as described by Anderson and colleagues for testing bone substitutes.

***Table 1*. Currently available animal models for the evaluation of biological responses to dental implants, categorized for species, implant dimensions and number of implants placed per animal**

Animal model	Implant dimensions (mm)		No. implants placed per animal	References
	Diameter	Length		
Tibia				
Rat	1–1.9	2–2.5	1	Wermelin et al. (2008), Kajiwara et al. (2005)
Guinea pig	1.8–2	5–7	1	De Smet et al. (2006), De Smet et al. (2008)
Rabbit	3–3.75	8	2	He et al. (2009), Meirelles et al. (2008), Susin et al. (2008)
Minipig	4.1	10	4	Buchter et al. (2005a, 2005b)
Dog	4–6	6–18	4	Coelho & Lemons (2009), Suzuki et al. (2009), Cook et al. (1992)
Goat	3.8–4.6	10–13	4	Vercaigne et al. (1998b), Shalabi et al. (2007), Schierano et al. (2005)
Sheep	6–6.5	15–18	5	Svehla et al. (2000, 2002)
Femoral condyle				
Rat	1.75–3	3.5–5	1	Schouten et al. (2009c), Peter et al. (2006)
Rabbit	3.25–3.75	4–7	2	Klokkevold et al. (2001), Susin et al. (2008)
Dog	6	9–16	2	Lind et al. (1996a, 1996b), Thomas & Cook (1985)
Sheep	3–13	5–130	2	Stadelmann et al. (2008), Heimann et al. (2004)
Goat	3.6–9	8–10	3	Schouten et al. (2009a), Caulier et al. (1995), Nikolidakis et al. (2009), Clemens et al. (1998), Hulshoff et al. (1997)

Based on the results of the aforementioned studies and the continuous need to refine animal models, the present study focused on the ability to place dental implants mono-cortically, i.e. inserting implants on top of the iliac crest down into the trabecular bone of the os ileum. This method has, as far as the authors know, has never been described before.

First, the iliac crests of two goat cadavers were studied with respect to anatomy and implant positioning. Additionally, the bone structure was evaluated and compared with that of the goat femoral condyle. Secondly, the model was validated by performing an *in vivo* experiment.

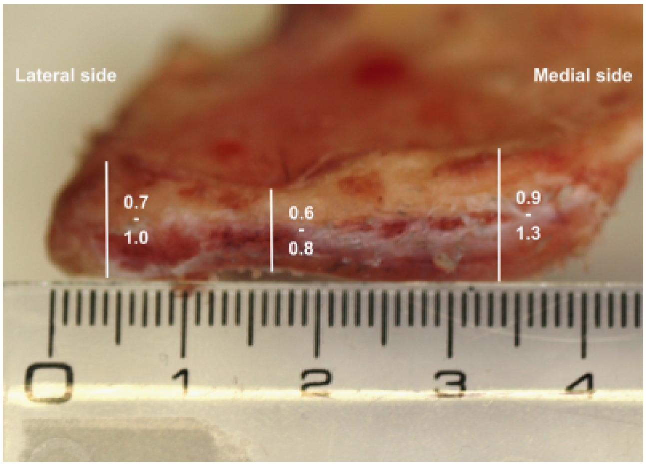

Fig. 1. Representation of the anatomical dimensions of the left iliac wing. The diameter varies along the iliac wing from 0.9 to 1.3 cm on the medial side, 0.6 to 0.8 cm on the inner side and 0.7 to 1 cm on the lateral side.

Feasibility of the iliac crest as an implantation model

Anatomy

As the anatomy of the iliac crest resembles more or less an hourglass, the width of the iliac wing measured ranged from 0.9 to 1.3 cm on the medial side, 0.7–1 cm on the lateral side and 0.6–0.8 cm in between. The total length of the iliac crest varied between 3.8 and 4.5 cm (Fig. 1).

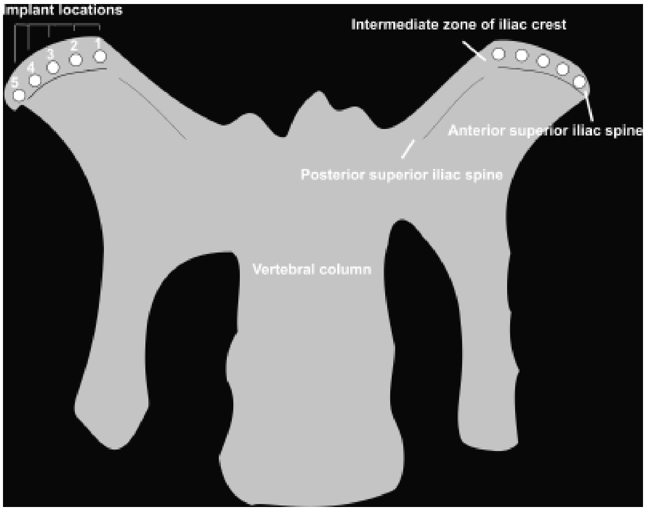

Fig. 2. Schematic representation of the pelvic of the goat. The numbered cavities represent the five available implant sites in the iliac crest.

Positioning the implants

Based on the anatomical dimensions, it was judged that in each iliac crest, five dental implants with a diameter of about 4 mm and a length of 13 mm can be placed monocortically. Ultimately, respecting an inter-implant distance of about 4 mm, a maximum of 10 implant sites per goat are available (Figs 2 and 3a). As an alternative, the implants can also be placed bicortically, resulting in four available implant sites (Fig. 3b).

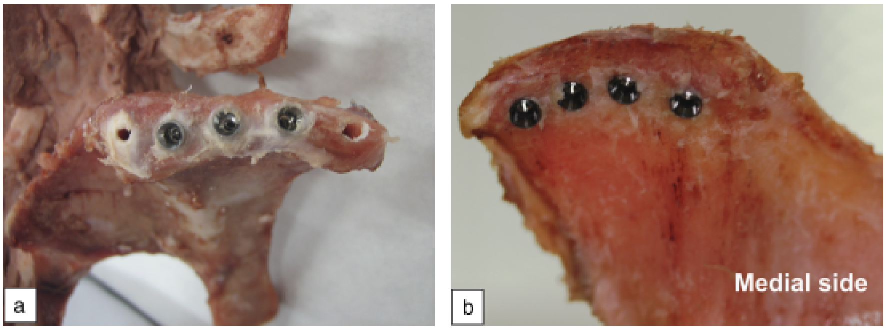

Fig. 3. (a) Representation of five available monocortical implantation sites in the right iliac wing of a goat. In the middle three bone cavities three dental implants were already placed. (b) Bi-cortical implantation of four dental implants in the left iliac wing of a goat.

Bone structure

To determine the bone structure of both the iliac crest and femoral condyle, micro-computed tomography (micro-CT) imaging was performed. Before scanning, small bone blocks were dehydrated in 70% ethanol and wrapped in Parafilm® (SERVA Electrophoresis GmbH, Heidelberg, Germany) to prevent drying during scanning. For a 3D analysis, the specimens were placed vertically onto the sample holder of a micro-CT imaging system (Skyscan 1072 desktop X-ray Micro-tomography System; Skyscan, Kontich, Belgium). 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. Finally, a 3D-reconstruction of the implant was obtained (3D creator software; Skyscan). For both the iliac crest and femoral condyle, an area of interest (5 × 5 mm) was defined, that corresponded to the site of implant placement, i.e. where the major part of the implant would be situated after implantation. Additionally, bone volume fractions (bone volume/total volume) were determined in the area of interest.

For the iliac crest, micro-CT images showed a well-defined trabecular structure, with the trabeculae oriented perpendicular to the outside borders and in a longitudinal direction in the center of the iliac crest (Fig. 4a–d), presenting a bone volume fraction of 21.7%. With respect to the bone structure in the femoral condyle, a more compact trabecular network with less defined trabeculae was observed, showing a bone volume fraction of 57.4% (Fig. 4e–h).

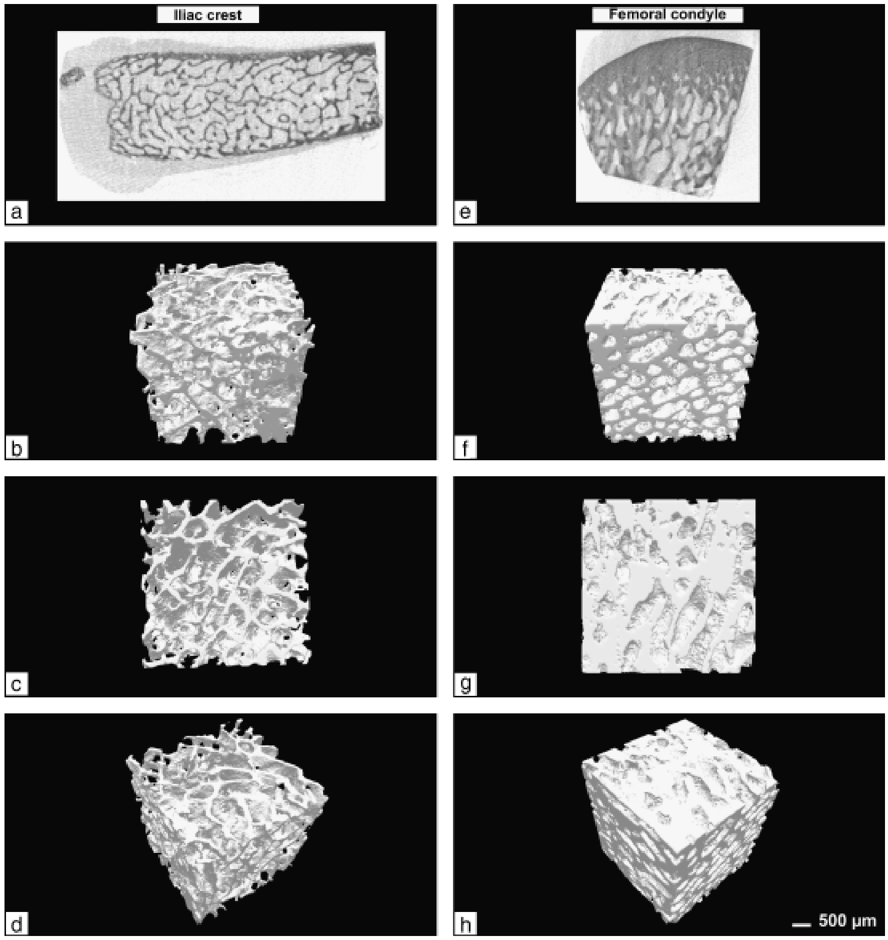

Fig. 4. (a, e) Two-dimensional cone beam reconstructed micro-CT image representing the trabecular bone structure in the iliac crest (a) and femoral condyle (e). (b–h). Three-dimensional reconstructed micro-CT image of the iliac crest (b–d) and femoral condyle (f–h). Represented is an area of interest (5 × 5 mm), corresponding to the part of the iliac crest where the screw-shaped part of the implant would be situated after implantation. (b–c) Represent a top (b), front (c) and side view (d) of the iliac crest, showing a well-defined trabecular structure. (f–h). Represent a top (f), front (g) and side view (h) of the femoral condyle, showing a compact trabecular network.

Validation of the iliac crest implantation model using an *in vivo* study

After evaluation of the feasibility of the model, an *in vivo* study was performed in four female Saanen goats (average age, 26–30 months; mean body weight, 50–60 kg), using similar dental implants as used in the cadavers. The detailed biological data of this *in vivo* study will be published separately.

First, each animal was immobilized in a ventral position, the pelvic areas were shaved and the anatomical structures were marked. A transverse skin incision was made, starting at the intermediate zone of the iliac crest (i.e. half way the posterior superior iliac spine and anterior superior iliac spine), subsequently processing toward the anterior superior iliac spine (i.e. from medial to lateral) on both sides of the vertebral column. The incision was continued through the underlying tissue layers down to the periosteum. Hereafter, the periosteum was undermined and lifted aside, exposing the iliac crest. For statistical reasons, in each iliac wing, only four implants (Dyna Dental Engineering BV, Bergen op Zoom, the Netherlands) were inserted, resulting in eight implant sites per goat (Fig. 5). Subsequently, the soft tissue layers and skin were closed with resorbable sutures (Vicryl® 4·0, Ethicon Products, Amersfoort, the Netherlands). To reduce postoperative pain, Finadyne® was administered for 2 days postoperatively. After 6 weeks of implantation, all four goats were euthanized with an overdose of Nembutal®, and the specimens were processed for histological analysis.

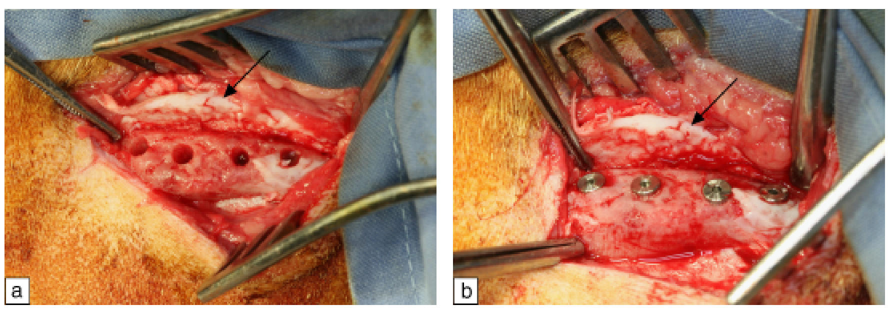

Fig. 5. (a–b) Representation of the right iliac wing showing four implant sites (a), and the implanted dental implants, with an inter-implant distance of 1 cm (b). In all animals, the top of the iliac crest was covered with a cartilaginous layer, which was shifted aside during surgical preparation (see arrows).

Histological preparations

After euthanasia of the animals, the iliac crests were harvested, excess tissue was removed and the specimens were fixed in 10% neutral buffered formalin solution and dehydrated gradually in ethanol solutions from 70% to 100%. Subsequently, the specimens were embedded in methyl methacrylate (MMA). Following polymerization, non-decalcified, 10-μm-thick, longitudinal sections of the implants were prepared (at least three of each implant), using a modified sawing microtome technique (Van der Lubbe et al. 1988), and subsequently stained with methylene blue and basic fuchsin.

General observations of the experimental animals

Throughout the experimental period, all four goats remained in good health and recovered successfully from the surgery. No postoperative bleeding, wound-healing complications or bone fractures were observed. After 6 weeks, all dental implants were still *in situ* (i.e. surrounded by bone), and no signs of inflammation or adverse tissue reactions were observed.

Descriptive histological evaluation

For a qualitative analysis of the bone response around the implants, histological evaluation was carried out using a light microscope (Axio Imager Microscope Z1, Carl Zeiss Micro imaging GmbH, Göttingen, Germany). Light microscopic examination of methylene blue/basic fuchsin-stained sections of the implants and surrounding tissue demonstrated variable amounts of bone inside and on top of the threads of the implants. The bone consisted of a well-defined trabecular network in the center of the iliac crest, with the trabeculae oriented perpendicular to the long axis of the implants. At the borders of the iliac crest, the bone appeared to be more compact, resembling cortical bone. An unexpected observation was the presence of a cartilaginous growth plate at the top of the iliac crest (Fig. 6).

Fig. 6. Histological representation of a dental implant inserted into the iliac crest of a goat after 6 weeks of implantation, showing a well-defined trabecular structure of the iliac bone and the presence of a cartilaginous growth plate at the top of the iliac crest.

Discussion

The aim of this study was to determine the feasibility and viability of the iliac crest in goats as a new implantation model, in order to serve as a promising alternative to already existing models. First, the iliac crests of two goats were studied *ex vivo* with respect to anatomy and implant positioning. Additionally, the bone structure was evaluated and compared with the femoral condyle. Thereafter, an *in vivo* experiment was performed to validate the suitability of the iliac crest model for the evaluation of the *in vivo* bone response to dental implants. The results of the present study show that by means of a rather simple, safe and reproducible surgical procedure, the iliac crest in goats can be approached and allows implantation of up to five dental implants per iliac crest. As such, and more specifically due to the symmetry of the iliac crest, and hence the possibility to use this model in a bilateral set-up, statistical comparisons between groups on either side of the iliac crest can be performed, which considerably reduces the use of laboratory animals and associated costs. In conclusion, the iliac crest model presented foresees in the primary goal of animal experiments in implantology, i.e. achieving reliable data on the biological performance of implants. Additionally, these biological data can be obtained using lesser number of experimental animals, and hence lesser costs.

Surgical approach

As the long-term clinical success of dental and orthopedic implants is still highly influenced by their physicochemical surface characteristics, research in the biomedical field is still focusing on implant surface modification to optimize the biological response. To study the biological response at the bone–implant interface, the femoral condyle model still is the most frequently used implantation model. Therefore, in the following discussion the femoral condyle model is used as a reference for comparison with the newly presented iliac crest implantation model.

Regarding the surgical approach, for both the medial and lateral femoral condyle model, locating the flat surface of the condyle suitable for implant placement is rather difficult. Moreover, in cases of bilateral implantation, i.e. operating both hind limbs, positioning of the animal in such a way that both condyles are easily approached, is very complex, nearly always resulting in repositioning of the animal during surgery, which is rather inconvenient and time-consuming (Lind et al. 1996b; Clemens et al. 1998; Nikolidakis et al. 2009). As demonstrated in the present study, the iliac crest implantation model does not entail these limitations. Animals can be immobilized in a ventral position, exposing both iliac crests at the same time. The anatomical dimensions of the iliac crest are easily recognized, making determination of the exact incision location rather simple. During the surgical procedure, no vital structures will be encountered and, as the iliac crest is fully exposed, the surgeon has a clear view where to create the bone cavities and place the implants. In view of this, the surgical procedure related to the iliac crest implantation model can be considered (i) simple, (ii) reproducible and (iii) safe. Even more important is the resultant short operating time, which leads to a short period of anesthesia, and thereby a quick recovery of, and less stress for the animals.

The validation of the iliac crest implantation model showed no postoperative complications. In literature also, no long-term complications are described for the femoral condyle model. However, for the short-term, occasionally transient swollen knees were observed due to edema or hematoma. Therefore, the Experimental Animal Ethical Committee from the Radboud University Medical Center (Nijmegen, the Netherlands) recently decided that bilateral implantation in the femoral condyles of goats is prohibited for future studies.

Anatomy, implant positioning and bone structure

Its large body size makes the goat highly suitable for implantation of multiple implants or implants of considerable size.

When the femoral condyle model is chosen, literature describes models in which a maximum of three implants per condyle can be placed (Schouten et al. 2009a; Nikolidakis et al. 2009). Because of the spheric aspect of the condyle, care must be taken during implantation to prevent contact between apical parts of the implants, consequently limiting the length of the implants. The results obtained in the present study showed that a maximum of five dental implants with a diameter of 4.2 mm could be placed in each iliac wing, resulting in 10 implant sites per goat. The implants were placed parallel to each other, on top of the iliac crest, between the outer and inner cortical layer of the os ileum. Consequently, the iliac crest model does not have restrictions regarding implant length. Besides goats, the iliac crest implantation model could also be used in alternative animal species, such as rabbits, mini pigs, dogs and sheep. The ultimate use of the iliac crest model in these species, however, requires feasibility studies before experimental use. Furthermore, due to differences in animal body size, implant diameters and the number of implants that can be placed, will differ accordingly.

For extrapolation of biological data, physiological similarities between the animal model and humans are required. In view of this, literature reports that goats have a metabolic and bone remodeling rate similar to that of humans (Spaargaren 1994). Qualitative analysis of the bone structure in the goat iliac crest showed a well-defined trabecular structure, with a different orientation of the trabeculae in the center of the iliac crest compared with the peripheral borders. Evaluation of the histological sections revealed that the trabecular network in the center of the goat iliac crest had a rather porous architecture, which was objectified by micro-CT analysis, showing a rather low bone volume fraction (21.7%) also. Micro-CT as well as histological analysis of the goat femoral condyle showed a less defined, but more compact trabecular network compared with the iliac crest. This was objectified by a twofold bone volume fraction compared with the iliac crest (57.4%). A previous study by Stadelmann, in which the bone volume faction of goat iliac crest biopsies was determined, corroborates these findings, as they found a bone volume fraction of 19% (Stadelmann et al. 2008). The fact that the bone volume fraction in the iliac crest is substantially lower than in the femoral condyle, makes the iliac crest highly suitable for testing the bone–implant interface in low quality bone.

It should be emphasized that in this study, only models that evaluate the bone implant interface in an unloaded situation were addressed. If loading aspects also need to be taken into account, intra-oral implantation models are preferred. Numerous load-bearing model set-ups have been performed in, for example, dogs, goats and monkeys (Caulier et al. 1995; Vercaigne

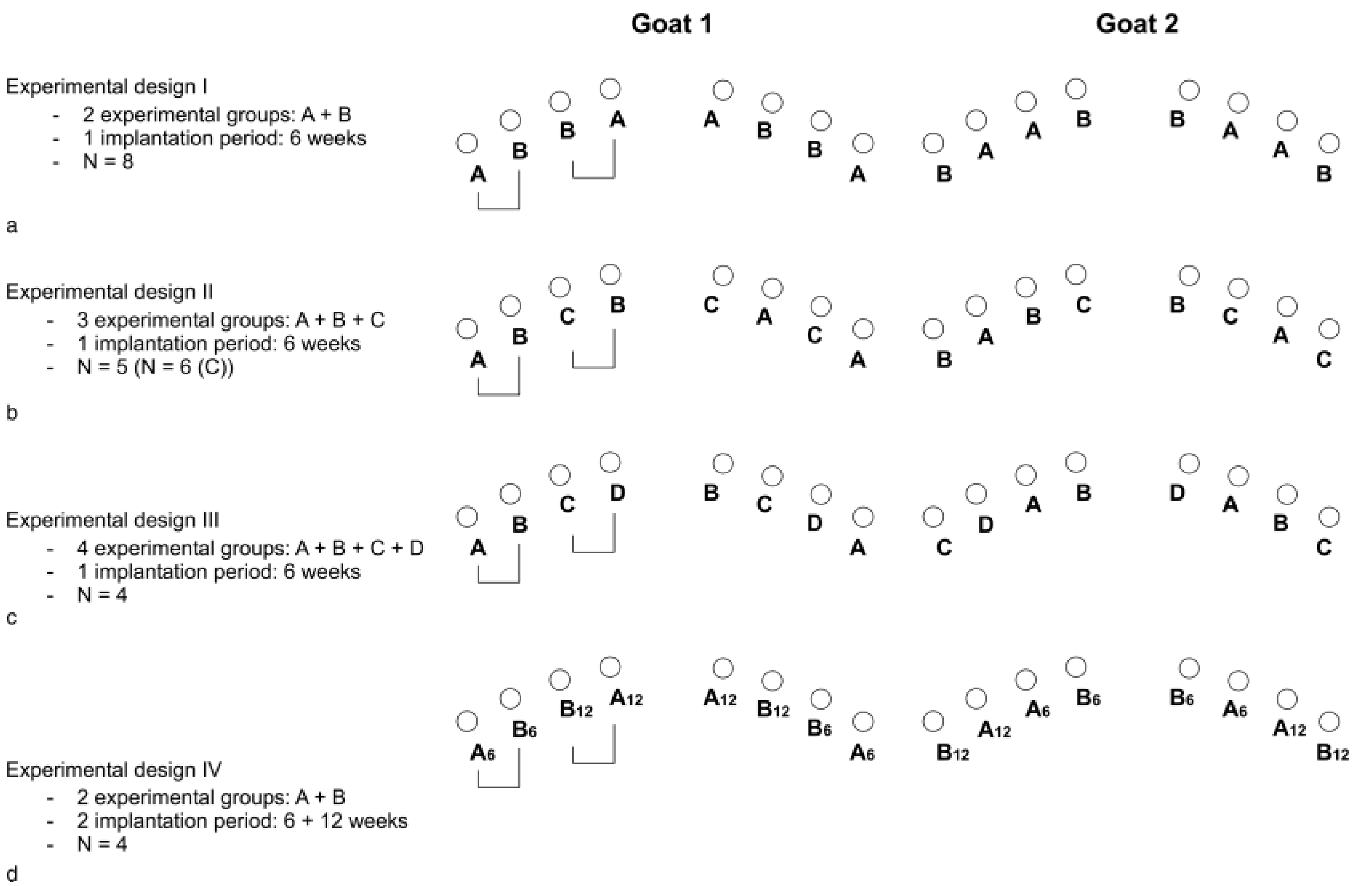

Fig. 7. Schematical representation of various Latin Square Randomization Schemes for two different experimental groups distributed over the left and right iliac wing of a goat (a–b). (a) Representation of the distribution of the implants in the *in vivo* study evaluating two different experimental groups and one implantation period. (b) Distribution of implants of three experimental groups and one implantation period. (c) Distribution of implants of four experimental groups and one implantation period. (d) Distribution of implants of two experimental groups and two implantation periods.

et al. 1998a; Carr et al. 2001; Vernino et al. 2002; Conner et al. 2003; Schliephake et al. 2009). A disadvantage related to these studies, however, is the fact that they are more expensive: the placement of implants needs to be preceded by molar extraction and subsequent healing.

Statistical evaluation

In the *in vivo* experiment described in the present study, the bone response to two experimental groups was evaluated, and therefore four dental implants were placed in each iliac crest. Placing only four implants was preferred over placing five implants, as it allows statistical comparisons of both groups within one side and one animal (paired left and paired right), instead of between opposing sides within one animal (paired left–right), resulting in a higher statistical power.

An experimental model should be designed in such a way that data can be compared within one animal and preferably within either side of the animal. Despite these precautions, due to external influences, such as (i) the health and general condition of the animal (quality of the bone), (ii) local differences between implant sites (trabecular bone, cortical bone), and (iii) surgical technique, variations might still occur. To balance/minimize such influences, specific randomization schemes can be applied for allocation of the implants. In the *in vivo* study performed in the present study, implants were distributed over the animals according to a Latin square randomization scheme (Navia 1977; Jansen et al.1996), which is a randomization scheme that guarantees control over experimental variation depending on implant location. Figure 7a displays the distribution of the implants of the two different experimental groups in the right and left iliac crest of two goats. Figure 7b–d display the distribution of implants for different experimental designs, showing the suitability of the iliac crest implantation model in various situations.

The fact that the bilateral use of the femoral condyle model is prohibited by the Experimental Animal Ethical Committee of the Radboud University Nijmegen Medical Center, increases the value of the bilateral iliac crest implantation model to a high extent, from a statistical point of view also.

In summary, the iliac crest is presented as a new implantation model suitable for evaluation of the osteogenic response to implant materials. It was demonstrated that in terms of surgical approach, anatomy and implant positioning, the iliac crest is advantageous over the femoral condyle model. By a relatively simple, safe, fast and reproducible procedure, bone defects were created in the iliac crest of goats allowing qualitative and quantitative analysis of large implants. Because of the ease of handling, the resultant pain and stress for the animals were rather low, and the recovery of the animals quick. Moreover, it was demonstrated that the goat iliac crest model allows the implantation of a maximum of 10 implants per animal and due to the bilateral implantation scheme, statistical comparisons can be performed using paired analysis on either side of the iliac crest, resulting in a very high statistical power. Consequently, by using the goat iliac crest as a model for the evaluation of the osteogenic response to implants, a reduced number of animals is needed for biological evaluation, making this model very promising for future use in *in vivo* experiments.

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