Animal Models Used in Testing the Biocompatibility of the Dental Implant – A Review

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Abstract

In order to investigate the interaction between the bone and the dental implant, in vivo tests are performed in accordance with the biocompatibility requirements. As in vitro tests do not provide sufficient data on the quality of the implants, the animal models are used because they allow a complete assessment of all the factors involved in the osseointegration process.

The aim of the paper is to provide data on the bone structure of animal species involved in dental research, highlighting the complexity of bone structure and osseointegration depending on the species. At the same time, the importance of selecting animal models is emphasized, in order to obtain the most accurate data for the development of dental implants and their use in humans. Following the research, essential data were obtained that helped to synthesize the bone characteristics of animals such as rats, rabbits, pigs, sheep or goats and relate them to the human bone skeleton. These data are useful in choosing an animal model when testing a new dental implant.

Keywords: animal model, bone, dental implant, osseointegration

Introduction

The success of applying a dental implant is influenced by factors that are dependent on both the type of device used and the individual. For several years, an attempt has been made to achieve an optimal interaction between the bone and the implant, aiming for it to be accepted by the body, to have as few adverse reactions as possible after the surgical process and to have an increased resistance to wear (Schmidt, 2001).

To verify that a dental implant is biocompatible and meets the safety requirements, it must be tested in vitro and in vivo. Cytocompatibility is the first step in assessing the toxicity of materials used in the manufacture of medical devices to characterize them in contact with bone cells. In vitro tests, moreover, are preliminary and are

performed to avoid unnecessary use of animals if the materials are cytologically unsuitable. The installation of a medical device in the body of an animal is the most effective way to assess local effects on surrounding tissues, and the biocompatibility standards contained in ISO 10993-6 set out the general considerations to be taken into account when testing (ISO 10993-6:2016 – Part 6).

The selection of the animals that can be used for testing the dental implants is one of the most important steps, because each animal has a different bone structure. The purpose of this review is to know the details regarding the macro and microstructure of the bone for each animal species used in the research of the bone-implant interaction.

The characteristics of dental implants that are used in the in vivo tests

Currently, there is a wide variety of dental implants, but when it comes to testing them on an animal model, one of the important factors that ought to be considered, is their size. It must be related to the size of the animal and especially their bones, so improper mounting or possible bone fractures, that would definitely result in research failure and unjustified use of animals, can be avoided. The sizes of the implants, recommended in various practical guidelines, that are used for the insertion in to the bone cortex, are closely related to the animal species. As to their integration, it is variable over time from one individual to another (Table 1), (ISO 10993-6:2016 – Part 6).

The vast majority of dental implants have a cylindrical shape, threaded, screw type, that is covered with various biomaterials. The purpose of these characteristics is to ensure an initial stability in the bone and later to favor the osseointegration (Almutairi *et al.*, 2018).

Animal Selection

Dental medical devices, before being used in dental practice, are tested by inserting them into the animal bone. The selection of the animal species must be carefully documented and justified, taking into account the physiological peculiarities of each animal but also the similarity with the human bone (Table 2). The factors that are taken into account, when deciding on the use of a particular animal species, are those related to the availability of animals, their cost, the maintenance conditions,

Table 1. Size of dental implants / animal species and the time required for osseointegration

Species	Implant length	Implant diameter	Required time for osseointegration
Rat	5 mm	1.6 mm	12-52 weeks
Rabbit	6 mm	2 mm	12-104 weeks*
Dog	12 mm	4 mm	12-104 weeks*
Sheep / Goat	12 mm	4 mm	12-104 weeks*
Pig	4.5 mm	2 mm	12-104 weeks*

*The time required for osseointegration varies depending on the material that is tested and the expected

Table 2. Similarities between human and animal bone (Bonucci *et al.*, 2014; Chu *et al.*, 2010; Roach *et al.*, 2003)

Species	Macroscopic similarities	Microscopic similarities	Age of bone skeleton maturation
Human	–	Lamellar bone with extensive remodeling	16-20 years
Rat	None	Primary bone throughout life	26 weeks
Rabbit	Minimal similarities	Primary bone with development of secondary osteons with the advancement in age the development of secondary osteons with age	8-11 months
Pig	Moderate similarities	Plexiform bone with transition to secondary osteon	2-4 years
Small ruminants	Moderate similarities	Plexiform bone up to 7-9 years of age (sheep) and secondary osteon (goat)	2 years
Dog	Similar	Plexiform bone with small osteon remodeling	18 months

their tolerance to the conditions of captivity, the existence of qualified personnel to handle the animals but also the suitability for surgery or the lifespan of the species that must be adequate to the study plan (Frisbie *et al.*, 2006; Zoetis *et al.*, 2003).

International Standard ISO 10993, Part 6 states that rabbits, dogs, small ruminants and pigs are used for testing the dental implants.

Rats

Even though they are not covered by ISO 10993-6, due to differences in bone structure (compared to humans), their size and limited number of devices for testing, recent studies show that this animal species is frequently used, probably due to their increased availability and of the fact that they are easy to handle and take care of. Attributes of the ideal animal model used in dental research include anatomical and physiological similarities with humans, so the rat can be used on a small scale in the dental medicine field, due to the presence of the anatomy of their incisors and relatively small areas of spongy bone (Elovic *et al.*, 1995; Jiang *et al.*, 2004; Lerouxel *et al.*, 2004). When used in studies, the area of choice for the implant placement is the mandible, femur and tibia (Table 3). To reduce the number of animals that will be used, it is preferred that the insertion of the implant, be performed on both limbs.

Rabbits

In medical research rabbits are used in more than 35% of the cases. Their advantage is that they are easy to handle and have a high availability rate, also the maturity of their bone system is reached relatively fast in comparison with other species, immediately after they reach sexual maturity at the age of 6 months (Pearce *et al.*, 2007). One of the reasons why this animal model isn't desired in the studies of oral mechanics or the osseointegration of dental implants, is the fact that the dental plaque is without roots and deeply embedded in

the mandibular bone structure, which also has few areas of spongy bone. Another aspect taken into account in extensive research projects, which involve testing a large number of implants, is that no more than 6 devices can be used on the rabbit, also their size must, obviously, be adapted to the size of the animal (ISO 10993-6:2016 – Part 6). From the histological point of view, the rabbit's long bone is very different from the human one, in the sense that it has a longitudinal vascular network that includes the osteon channels, being found in the medullary canal, but also in the periosteum. Bone mineral density in the femoral or tibial cortex is lower, probably due to the poorly developed Haversian system (approx. 700 g/cm², femoral bone density and 973.6 g/cm² mandibular bone density). The maximum average, for the diameter of the osteon described by Martiniakova *et al.* (2005), was 223.79+47.69 µm with a minimum average diameter of 50.79+9.71µm (Martiniakova *et al.*, 2005). Compared to other animal models, the rabbit's skeletal system shows a rapid response (Castaneda *et al.*, 2006) to the actions of an external stimulus, therefore, along with the prior mentioned disadvantages, the rabbit, as an animal model, isn't preferred in medical research, because it's use can interfere with the extrapolation of the final results.

Pigs

Pigs are often used for testing of dental implants due to their bone structure that resemblance in many ways the human one and the large number of devices that can be put on a single animal (Bagi *et al.*, 2011). One of the disadvantages of the use of this animal model is their high growth rate and the difficulty in handling them caused by their reactive behavior or low stress resistance. To eliminate these inconveniences, mini-pig lines have been created, and among the most used

Table 3. Cortical and spongy bone, parameters obtained from rats by microCT analysis (Bagi *et al.*, 2011)

	Proximal femoral diaphysis	Mandible
Tissue volume (mm ³)	2.66 ± 0.12	0.97 ± 0.06
Bone volume (mm ³)	1.01 ± 0.03	0.60 ± 0.14
Bone density (g/cm ²)	1117.6 ± 9.5	1024.3 ± 42.5
Cortical layer thickness (mm)	0.44 ± 0.03	–
The volume of the bone marrow	1.64 ± 0.12	–

in medical research are Yucatan, Lee-Sung and Göttingen (Coelho *et al.*, 2018).

From the anatomical, morphological and the healing and remodeling capacity point of view (An and Friedman, 1999), the pig bone is similar to the human one, although, microstructurally, the trabecular network is much denser in pigs than in humans (Mosekilde *et al.*, 1987). Similarities such as lamellar structure, density and concentration of minerals in the bone, compared to humans (Aerssens *et al.*, 1998; Schierano *et al.*, 2005), make the pig a good study model for the osseointegration process of dental implants, especially since their tibia is considered the equivalent of the human mandibular bone (Del Fabbro *et al.*, 2017).

A review of miniature pigs, shows the concordance between the dental characteristics of this species and those of humans (Kantarci *et al.*, 2015), so natural gingivitis can be observed from the age of 6 months and is manifested by gingival edema, percussion bleeding and the existence of bacterial plaque and from the age of 16 months, pigs can even develop periodontal diseases (Wang *et al.*, 2007). This animal model is often used in experimental peri-implantitis studies where aspects such as bone resorption, gum inflammation, loss of attachment (implant) and microbiota modifications are evaluated (Hickey *et al.*, 1991).

Small ruminants (sheep / goats)

Sheep or goats are not that used for testing the dental implants, compared to other species, although the macroscopic bone structure is close to that of humans. Histologically, the bones of small ruminants are different, the osteon has a diameter of 100µm and contains at least two central blood vessels. The age of the animal greatly influences the bone structure, the primary “embryonic” bone appears strongly vascularized and poorly organized in terms of mineralization (Lopez-Pliego *et al.*, 2018). After the age of 4, the sheep have a plexiform bone structure that includes lamellar bone tissue interposed with the vascular plexuses, similar to a sandwich. At 7-8 years of age, the Haversian system reshapes, becoming much more prevalent, especially in the femur and humerus (Newman *et al.*, 1995).

Bone density and strength is much higher in small ruminants, the femoral trabecular bone being twice as dense as in humans, but there are studies that note that the density of the bone

assembly differs depending on the anatomical region. In dental research, the bones of interest are represented by the femur and tibia, where several implants can be mounted because the bone remodeling and the healing potential are great. When deciding on the use of small ruminants in osseointegration studies, it should not be overlooked the fact that the bone changes during the cold season, when bone regeneration is depressed, which could influence the objectives of the experiment (Wancket, 2015).

Dogs

The use of dogs in biomedical research has recently been hit by certain ethical issues due to the fact that this species is considered a pet. Dogs are frequently used in dental studies and are a valuable model in the study of peri-implantitis due to their susceptibility to spontaneous periodontitis (Dard, 2012).

Canine and human bone have many similarities that include weight, density, organic and inorganic composition. Secondary osteon formation, epiphyseal fusion at maturity, intracortical remodeling, or bone loss in old age are other aspects of similarity to humans (Bonucci *et al.*, 2014). The difference between human and canine bone is the speed of bone remodeling, which in dogs is higher in the trabecular component. Regarding this aspect, there are variations both depending on the anatomical region (eg lumbar vertebrae have a bone transformation rate of almost 200% / year in young Beagle dogs) but also between individuals, the average value of the degree of bone regeneration being however about 100% (Liebschner, 2004). The canine bone microstructure is mixed and includes secondary osteonal bone in the center of the cortex, and adjacent to the periosteum and endosteum is the plexiform bone that is formed quickly and in large quantities, providing increased mechanics and support (Wang *et al.*, 1998; Kimmel and Jee, 1982).

In studies related to dental implants, Beagle is the accepted breed, and as an anatomical mounting region, the femur is preferred, even if many publications describe the intra-oral region, in the working method, when following the processes of osseointegration, peri-implantitis or tissue healing after experimental treatments (Vignoletti *et al.*, 2012).

The data obtained from human research have the highest scientific value but in studies related

to dental implants, the human model offers the disadvantage of variations such as: patient's age, health, number of the tested subjects or the need for histological evaluation of the samples. All these factors do not provide statistical relevance and make the evaluation process of a medical device difficult.

Conclusions

In dental research, animal experimentation is necessary and may provide data that cannot be extrapolated from human studies, due to the impossibility of harvesting tissues required for microscopic analysis. No species meets the ideal requirements, but an understanding of the bone differences in macroscopic, microscopic, and bone remodeling structure can direct the results of in vivo experiments.

The purpose of selecting an animal model in dental implant research, is to obtain sufficient information on the body's response to a foreign body, wound healing around implants, tissue architecture, or the mechanisms of periodontal disease.

Of the species described, the dog has the most similar bone structure to humans, which is why it is used in over 45% of studies, followed by the rabbit with a frequency of use of 35%. Small ruminants and pigs, whose bones are close to human and do not have ethical inconveniences such as dogs, raise issues of availability, maintenance and handling.

As implant development and clinical use are on the rise, it is important to conduct studies to evaluate them. Animal models can provide strong support in such studies, as long as the sample is large enough to generate statistically useful data and small enough to prevent unnecessary use of animals.

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References

1. Aerssens J, Boonen S, Lowet G (1998). Interspecies differences in bone composition, density, and quality: Potential implications for in vivo bone research. *Endocrinology*, 139:663–670.
2. Almutairi AS, Walid MA, Alkhodary MA (2018). The effect of osseodensification and different thread designs on the dental implant primary stability. *F1000Res*, 7:1898.
3. An YH, Friedman RJ (1999). Animal selections in orthopaedic research. In: An YH, Friedman RJ, ed. *Animal Models in Orthopaedic Research*. Boca Raton, FL: CRC Press LLC:39–57.
4. Bagi MC, Berryman E, Moalli MR (2011). Comparative Bone Anatomy of Commonly Used Laboratory Animals: Implications for Drug Discovery. *Comp Med*, 61(1): 76–85.
5. Bonucci E, Ballanti P (2014). Osteoporosis—bone remodeling and animal models. *Toxicol Pathol*, 42(6): 957–969.
6. Castaneda S, Largo R, Calvo E, Rodriguez-Salvanes F, Marcos ME, Diaz-Curiel M, Herrero-Beaumont G (2006). Bone mineral measurements of subchondral and trabecular bone in healthy and osteoporotic rabbits. *Skeletal Radiol*, 35: 34–41.
7. Chu CR, Szczodry M, Bruno S (2010). Animal models for cartilage regeneration and repair. *Tissue Eng Part B Rev*, 16(1): 105–115.
8. Coelho PG, Pippenger B, Tovar N, Koopmans SJ, Plana NM, Graves DT, Engebretson S, van Beusekom HMM, Oliveira PGFP, Dard M (2018). Effect of Obesity or Metabolic Syndrome and Diabetes on Osseointegration of Dental Implants in a Miniature Swine Model: A Pilot Study. *J Oral Maxillofac Surg*, 76(8):1677–1687
9. Dard M (2012). Methods and interpretation of performance studies for dental implants. In: Boutrand, ed. *Biocompatibility and Performance of Medical Devices*. Oxford, UK: Woodhead Publishing Limited, 308–344.
10. Del Fabbro M, Taschieri S, Canciani E, Addis A, Musto F, Weinstein R, Dellavia C (2017). Osseointegration of Titanium Implants With Different Rough Surfaces: A Histologic and Histomorphometric Study in an Adult Minipig Model. *Implant Dent*, 26(3): 357–366.
11. Elovic RP, Hipp JA, Hayes WC (1995). Ovariectomy decreases the bone area fraction of the rat mandible. *Calcif Tissue Int*, 56: 305–310.
12. Frisbie DD, Cross MW, McIlwraith CW (2006). A comparative study of articular cartilage thickness in the stifle of animal species used in human pre-clinical studies compared to articular cartilage thickness in the human knee. *Vet Comp Orthop Traumatol*, 19(3): 142–146.
13. Hickey JS, O'Neal RB, Scheidt MJ, Strong SL, Turgeon D, Van Dyke TE (1991). Microbiologic characterization of ligature-induced peri-implantitis in the microswine model. *J Periodontol*, 62: 548–553.
14. ISO 10993-6:2016. Biological evaluation of medical devices — Part 6: Tests for local effects after implantation
15. Jiang G, Matsumoto H, Yamane J, Kuboyama N, Akimoto Y, Fujii A (2004). Prevention of trabecular bone loss in the mandible of ovariectomized rats. *J Oral Sci*, 46:75–85.
16. Kantarci A, Hasturk H, Van Dyke TE (2015). Animal Models for Periodontal Regeneration and Peri-Implant Responses. *Periodontol* 2000, 68(1): 66–82.
17. Kimmel DB, Jee WS (1982). A quantitative histologic study of bone turnover in young adult beagles. *Anat Rec*, 203: 31–45.
18. Lerouxel E, Libouban H, Moreau MF, Basle MF, Audran M, Chappard D (2004). Mandibular bone loss in an animal

- model of male osteoporosis (orchidectomized rat): a radiographic and densitometric study. *Osteoporos Int*, 15: 814–819.
19. López-Pliego EM, Mora-Macías J, Giráldez-Sánchez MA, Domínguez J, Reina-Romo E (2018). Histological Study of the Docking Site After Bone Transport. Temporal Evolution in a Sheep Model, 49(11): 1987-1992.
 20. Martiniakova M, Omelka R, Chrenek P, Ryban L, Parkanyi V, Grosskopf B, Vondrakova M, Bauerova M (2005). Changes of femoral bone tissue microstructure in transgenic rabbits. *Folia Biol (Praha)*, 51: 140-144.
 21. Mosekilde L, Kragstrup J, Richards A (1987). Compressive strength, ash weight, and volume of vertebral trabecular bone in experimental fluorosis in pigs. *Calcif Tissue Int*, 40: 318–322.
 22. Newman E, Turner AS, Wark JD (1995). The potential of sheep for the study of osteopenia: current status and comparison with other animal models. *Bone*, 16: 277S-284S.
 23. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG (2007). Animal Models for Implant Biomaterial Research in Bone: A Review. *Eur Cell Mater*, 13: 1-10.
 24. Roach HI, Mehta G, Oreffo RO (2003). Temporal analysis of rat growth plates: cessation of growth with age despite presence of a physis. *J Histochem Cytochem*, 51(3): 373–383.
 25. Schierano G, Canuto RA, Navone R (2005). Biological factors involved in the osseointegration of oral titanium implants with different surfaces: A pilot study in minipigs. *J Periodontol*, 76: 1710–1720.
 26. Schmidt C, Ignatius AA, Claes LE (2001) Proliferation and differentiation parameters of human osteoblasts on titanium and steel surfaces. *J Biomed Mater Res* 54: 209-215.
 27. Vignoletti F, Abrahamsson I (2012). Quality of Reporting of Experimental Research in Implant Dentistry. Critical Aspects in Design, Outcome Assessment and Model Validation. *J Clin Periodontol*, 39 (Suppl) 12: 6-27.
 28. Wancket LM (2015). Animal Models for Evaluation of Bone Implants and Devices: Comparative Bone Structure and Common Model Uses. *Vet Pathol*, 52(5): 842-50.
 29. Wang S, Liu Y, Fang D, Shi S (2007). The miniature pig: a useful large animal model for dental and orofacial research. *Oral Dis*, 13: 530–537.
 30. Wang X, Mabrey JD, Agrawal CM (1998). An interspecies comparison of bone fracture properties. *Biomed Mater Eng*, 8(1): 1–9.
 31. Zoetis T, Tassinari MS, Bagi C (2003). Species comparison of postnatal bone growth and development. *Birth Defects Res B Dev Reprod Toxicol*, 68(2): 86–110.