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CONSIDERATIONS REGARDING THE USE OF EXPERIMENTAL ANIMAL MODELS IN DENTAL MEDICINE - A LITERATURE REVIEW

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Abstract

The purpose of the study is to document in the specialized literature, the importance of using experimental animal models in dental medicine. For this study we reviewed 41 abstracts of scientific papers and 81 in-extenso articles, using Web of Science Core Collection and PubMed databases. Additionally, a search in 5 specialized books was performed. Of these scholarly papers, 67 were considered to be relevant for this study. Even though the prevalence of these studies is decreasing due to the new legislative restrictions, the scientific results obtained with the use of these models in medical research are evident. The most relevant experimental animal model for scientific documentation of oral rehabilitation is the dog, both for dental implant surgery research and for the study of biomaterials used in the treatment of periodontal disease. The monkey is considered to be of excellent relevance for the evaluation of healing after periodontal treatments.

Key words: *experimental animal model, oral rehabilitation, dental implant*

INTRODUCTION

Experimental research involves choosing an experimental model appropriate to a certain predetermined scientific purpose.
The use of animals for experimental purposes is a fairly old scientific practice, which is still under development due to its advantages. For example, in dental medicine, many studies were performed using experimental models: research regarding dental implants osseointegration, epithelium and connective tissue attachment to dental implants, integration of bone grafts or studies for evaluation of pulpal inflammation.
The number of animals used in experiments remains high even in the third millennium. In Germany, for example, in 2001, approximately 2,126,000 animals were used in experiments (Sălăvăstru, 2014).
Similarities regarding embryological development and morphological resemblances in animals and humans are the basis of experimental studies.
Experimental animal models were recommended after performing histological and immunohistochemical studies that showed their possible clinical efficacy. In this regard, fragments of different tissues were collected from the experimental specimens, and then the pieces containing the areas of interest were analyzed immunohistochemically and histologically. Samples processing for the immunohistochemical and histological analysis must be in accordance with the legislation in force and with the recommendations of the medical practice guidelines for Anatomical pathology (Poll, 2015).
Also, the development of experimental models must be in accordance with the provisions of the Council of Europe Directive no. 86/609/ EEC on the protection of animals used for experimental or other purposes, with the Fifth Report on the statistics on the number of animals used for experimental purposes in EU Member States, ECA SEC 1455, Brussels (2007), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and with the regulations in force and with other regulations.
With the help of scientific literature data, the study presents a series of observations regarding the role of experimental animal models in scientific documentation of oral rehabilitation.

For experimental research in dental medicine, mainly for research related to periodontics and implant dentistry, it is recommended to use larger animal models and adult animals with a slower growth rate. Bones similar to the human maxilla and mandible, in what concerns the ratio of compact to spongy components, are preferred for the study of dental implant osseointegration (Babuska, 2016).

MATERIALS AND METHODS

A systematic search in the scholarly literature was conducted, in order to identify studies on the use of experimental animal models in scientific research in the field of dental medicine. 41 abstracts of scientific works and 81 in-extenso articles were accessed using Web of Science Core Collection and PubMed databases and the following key words: experimental animal model in dental medicine. Additionally, a manual search in five specialized books was performed. Of these scholarly papers, 67 were considered to be relevant for this study.

For the reasons presented above, this study analyzed in particular bibliographic references relevant for the use of experimental canine models.

RESULTS AND DISCUSSIONS

An animal model is defined as a non-human living animal with an inherited naturally acquired or induced pathological process or lesion allowing for the resolution of a research hypothesis and resembling a similar condition in the target human species (Hau et al., 1989).

In dental research, the animal models have particular applications for the study of periodontal disease and dental implant treatment. Initially, the pathogenesis of oral diseases, including experimental periodontitis, was aimed to be explored (Staubli et al., 2019). In recent years, animal experiments have been applied with respect to the performance of dental implants or the pathogenesis and therapy of peri-implant diseases (Staubli et al., 2019; Ericsson et al., 1996).

Small animals were rarely used as experimental models in dental medicine because of their oral, maxillary and mandibular anatomy which is different from the one of the human species. Consequently, small animal models have reduced clinical utility for dental medicine.

In the accessed references, we did not find examples of studies on **mice** that analyzed the dental implant integration at the level of the maxilla and mandible, the field of interest in this regard being not the intraoral bones, but the bones of the lower limb, such as the femur.

Because **the rat** jaws and teeth are small, the rat is mainly recruited for research on dental implants placed into the calvaria, the tibia, or the femur (Dard, 2012). The author reveals that only few studies dealt with intra-oral placed implants, and they described mini-implants placement into jaw bones, rather than implants placed for teeth replacement (Dard, 2012). Trabecular bone growth was examined around titanium mini-implants placed in rat femurs (Shimizu-Ishiura et al., 2002). Haga and coworkers are the ones who documented the bone formation and maturation around dental implants placed in the rat maxilla (Haga et al., 2009).

The rat is the most extensively studied rodent for the pathogenesis of periodontal diseases. A considerable difference exists between the human species and the rat, in that the rat is extremely resistant to periodontal disease (Struillou et al., 2010).

The gilded hamster (*Mesocricetus auratus*) remains the most interesting model for immunological research (Struillou et al., 2010).

A more systematic use of small animal models (rat and hamster) is recommended for future research on surgical treatment of periodontal disease (Struillou et al., 2010).

We have found only two publications that described studies in which **the cat** was used as an experimental animal for dental medicine research purposes (Takahashi et al., 2005; Silva et al., 2012).

The rabbit, which represents the animal of choice in about one-third of all musculoskeletal studies (Neyt et al., 1998), is also commonly used in implant dentistry (Dard, 2012). The rabbit was used as ‘pre-translational animal model’ in implant dentistry, for assessing dental implant designs and materials prior to testing them in a larger animal (Dard, 2012).

The rabbit has mainly been used for testing biomaterials or for investigating the treatment of peri-implantitis (Struillou et al., 2010).

In comparison to other species, such as primates, the rabbit has a faster bone turnover with significant intracortical, haversian remodeling (Pearce et al., 2007; Dard, 2012; Mapara et al., 2012).

Bone healing after guided tissue regeneration (well-known method in periodontal surgery), was studied on rabbit tibia model (Aaboe et all., 1994). Bone healing after application of PerioGlas (silicate-based synthetic bone augmentation material) in surgically created defects adjacent to titanium plasma-sprayed dental implants was studied on rabbit tibia model (Johnson et al., 1997). Bone regeneration promoted by porous bone mineral and biologically active glass (materials used for achieving alveolar bone augmentation and periodontal regeneration) was assessed on rabbit radius model (Schmitt et al., 1997). Although long bones, like tibia and radius represent interesting models for investigating the bone healing, they can not replicate the anatomical and physiological particularities of the maxilla, the mandible and of the alveolar bone (Struillou et al., 2010).

The accessed publications showed us that the number of experimental models that used sheep or goat for assessing the integration of dental implants is increasing, due to the dimensions of the maxillary and the mandibular bones in these animals, which are similar to those in the human species. However, there are no recent publications available using sheep as experimental animal model for research in periodontics (Struillou et al., 2010).

The swine is one of the most used animal species for translational purposes in pharmaceutical research. Over the last two decades, it has been positioned as a candidate among other species for use in musculo-skeletal surgical investigations (Dard, 2012).

The swine has bone remodeling processes similar to humans, comprising both a dense trabecular network and intra-cortical remodeling and it shows similarities in bone mineral density and bone mineral concentration to human bones (Mosekilde et al., 1987; Mosekilde et al., 1993; Aerssens et al., 1998). The miniature pig (micro- or minipig) offers several advantages over the domestic swine for dental implant research purposes. The advantages are mainly related to handling, housing and administering anaesthesia (Dard, 2012). The size, shape and anatomy of the minipig mandible and the movement of the temporomandibular joint in minipig are similar to those of humans (Dard, 2012).

Monkeys have the advantage of being philogenetically similar to humans (Struillou et al., 2010). The structure of the periodontium is also histologically similar to that observed in humans (Struillou et al., 2010). The inflammatory response to periodontal disease is quite similar to that found in humans: connective tissues are infiltrated by plasma cells, lymphocytes and neutrophils (Struillou et al., 2010). In some species, such as squirrel monkeys and marmosets, there is very limited inflammatory infiltrate. This major difference from humans makes them inappropriate models for studying the pathogenesis of periodontitis (Page and Schroeder, 1982; Struillou et al., 2010). The literature describes the monkey as an adequate experimental animal model for implant dentistry research, because of its oral healing characteristics similar to those of humans (Dard, 2012). However, clear ethical considerations limit the use of this animal model for surgical research purpose (Dard, 2012).

Monkey experimental models have been used for periodontal healing and biomaterials investigations (Drury et al., 1991; Ling et al., 1994; Sculean et al., 1997; Karatzas et al., 1999; Sculean et al., 2000) and evaluation of bone remodeling around loaded dental implants (Piattelli et al., 1998; Scarano et al., 2000; Piattelli, et al., 2003). Macaca fascicularis is the most used species for research on periodontal and dental implant surgery.

As *Macaca fascicularis* has the same dental formula as human, all the teeth can be used, which makes it possible to obtain an important number of test sites, with a limited number of animals (Struillou et al., 2010).

First introduced in the 1960s for research in periodontics, **the dog** has remained popular as model for studies on periodontal surgery. In this field, the studies address both spontaneous and experimental periodontitis in order to understand the etiopathology of the disease, its semiology, and the mechanisms of periodontal destruction and healing (Hennet, 1999).

Based on history and its previously broad use, one of the most preferred animals for research related to implant dentistry is *the Beagle dog* (Dard, 2012). However, this animal is highly affected by spontaneous periodontitis (Kortegaard et al., 2008; Dard, 2012).

Experimental animal models are useful for evaluating the behavior of biomaterials and the success of clinical procedures, as *in vitro* models cannot replicate the complexity of the human anatomy. It would be ideal that an experimental model for the study of the integration of bone autografts to have, embryologically and morphologically, similar biological traits in the donor and recipient areas (Poll et al., 2018; Isaksson, 1992; Klinge et al. 1992; Poll et al., 2018).

Research in dentistry has described different experimental animal models, such as the experimental model using rabbit as specimen (Atiya et al., 2014), the experimental model using rat as specimen (Korn et al., 2014; Levy et al., 2013), the experimental model using goat as specimen (Zou et al., 2012), the experimental model using pig as specimen (Ogunsalu et al., 2011), the experimental model using cat as specimen (Silva et al., 2012).

Other authors used the dog as model for experimental research in order to study bone regeneration in the case of autografts applied at the maxillary level (Pourebrahim et al., 2013; Oryan et al., 2014; Poll et al., 2018; Nimigean et al., 2019).

In the last decade many experimental studies on animal models were developed in order to increase the long-term performances of dental implants. These studies have shown that larger segments of bone autografts ensure a better conservation of bone volume and maintain bone height eight weeks after being applied (Ogunsalu et al., 2012; Ogunsalu et al., 2013; Kon et al., 2014).

Bone healing is a complex biological phenomenon that takes place both during the growth of the body and during its development stages, as well as in certain bone modeling, remodeling and repair processes. The necessary conditions for healing after surgery are mainly represented by: adequate blood supply, lack of connective tissue at the interface, and primary stability of the grafts (Poll, 2015).

On such experimental models, the integration of dental implants and autogenous bone grafts can be further studied through histopathological and immunohistochemical investigations, like are studied, for example, on dental pulp models the histological and immunohistochemical changes of pulp tissue exposed to different biomaterials (Nimigean et al., 2016; Nimigean et al., 2018; Nimigean et al., 2019; Tuculina et al., 2013).

It can be stated that the immunohistochemical assessment of the integration of mandibular autografts applied in maxillary bone defects, represents a valuable technique for the evaluation of initial and early phases of healing, statement similar to the conclusions of other authors (Schwarz et al., 2007). However, for the histological evaluation to be relevant, an increased resolution is required in order to distinguish the qualitative differences of the tissues, as other authors also have shown (Friedmann et al., 2014).

Immunohistochemical investigations regarding the relevance of experimental animal models have been less mentioned in the medical literature. As other studies showed, immunohistochemical investigations are rare or absent even in clinical research on certain infrequent pathological conditions (Vija et al., 2014).

The integration of the bone graft in the receiving area also depends heavily on its adequate revascularization, as it is independent of the vascular support of the receiving area (Elsalanty and Genecov, 2009).

The proliferation of bone cells is responsible for tissue regeneration, and osteocyte survival in the grafted areas depends directly on the blood supply and on the vitality of the periosteum (Salgado et al., 2011).

Particular morphologic patterns both at vascular level and at bone level (such as the relation of the mandible to the maxillae) might suggest predisposition to certain disorders and complications (Enache et al., 2010; Nimigean et al., 2018).

The experimental animal models are relevant for the study of periodontal disease, dental implants, biomaterials and new regenerative strategies in dental medicine (Struillou et al., 2010).

The relevannce of different animal models for research on periodontal treatment and dental implant surgery is presented in the Table 1 (Struillou et al., 2010).

Table 1. The relevance of different animal models for research on periodontal treatment and dental implant surgery (Struillou et al., 2010)

Species	Relevance according to research topic	
	Biomaterials for periodontal treatment	Dental implant surgery
Non-Human Primates	Excellent	Good
Dog	Excellent	Excellent
Minipig	Good	Medium
Rabbit	Medium	Medium
Rat	Medium	Low
Hamster	Low	Low

Experimental animal models are essential to understanding the origin and evolution of the periodontal diseases pathology in humans. The most commonly used animal model in periodontal research is the dog, due to the reproducible critical-sized defects. Many experimental studies on gingivitis and periodontitis have been conducted in dogs. The Beagle is one of the most commonly used due to its size and its extremely cooperative temperament (Struillou et al., 2010).

Studies in dogs have also reported research on mesenchymal stem cells and tissue engineering in the treatment of periodontal disease (Struillou et al., 2010).

The only criterion for the choice of a certain experimental research topic is its relevance, which cannot, however be judged in the short-term. A concept to systematically assess the relevance of the in vitro tests must be developed in order to increase quality and to finally achieve an evidence-based biomedical research (Gruber and Hartung, 2004).

An important role is attributed to the experimental salivary models, which do not have legislative restrictions, for example, Stefanescu et al. 2011, “Salivary monitoring of hormone levels has many advantages over the more conventional serum/plasma analysis”.

The use of experimental animal models in scientific research favors obtaining relevant and useful information for the human species.

However, experimental models are limited by scientific constraints and due to increased regulatory constraints (Knight, 2008; Leist et al., 2008; Lilienblum et al., 2008).

Experimental animal models have been used in dental and peri-implant research and have been a subject of debate in recent years (Staubli et al., 2019). Critical remarks have been published with respect to the transposing of research data derived from animals to the pathogenesis and therapy of human diseases and with respect to the ethical considerations on using experimental animal models for biomedical research (Staubli et al., 2019).

Like any chronic disease, periodontitis is a multifactorial pathological condition. The onset and progression of the periodontal disease are caused by the infection with a pathogenic oral biofilm. The composition of the oral biofilm is highly variable, showing intra- and interindividual diversity and it is affected by several behavioral factors, like nutrition and tobacco use and by local environmental factors, like the quality, the extent and the material of dental prostheses (Staubli et al., 2019). Moreover, scientific evidence indicates education and lower socio-economic status as risk factors for periodontal disease (Rodriguez et al., 2017). It is difficult, even impossible, to simulate most of the variables listed above in experimental animal models. Therefore, it is appreciated that animal experiments do not provide direct evidence relevant for human periodontal or peri-implant diseases (Staubli et al., 2019). The benefit for understanding pathogenesis or directions for therapy of human diseases needs to be critically defined for each experiment and for each animal used (Staubli et al., 2019).

Furthermore, a high standard of analysis and reporting of data obtained from animal research and adherence to quality guidelines such as the ARRIVE (animal research: reporting in vivo experiments) guideline are required to reduce the risk of bias derived from animal experiments (Kilkenny et al., 2010; Staubli et al., 2019).

A reduction in the number of experimental studies on animals for dental medicine research purposes has been registered during the last decade. This is in accordance with the implementation of the 3Rs principle - replacement, reduction, and refinement of the use of animal models in biomedical research (Pasupuleti et al., 2016).

CONCLUSIONS

The experimental animal models are useful for understanding the oral pathology in humans.
The literature review is beneficial in evaluating the relevance of experimental animal models for dental medicine research purposes, because the different indications regarding their use require tailored solutions.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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