Review Article

AHB Advances in Human Biology

Adv Hum Biol. 2015;5(2):1-16.

Validity and Variability of Animal Models Used in Dentistry

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ABSTRACT

Background: Animal models have contributed to dental literature for several decades. The major aim of this review was to outline tooth development stages in mice, and attempt to addressing potential strain differences. A literature review was performed using electronic and hand-searching methods for the animal models in dentistry with special emphasis on mice and dentistry. Root canal development in both C57BL/6 and BALB/c strains were investigated. There are a number of published reports regarding the morphogenesis and molecular reaction and maturation stages of mice molars. We observed some similarity between the mice and human odontegeneis as primary factor for tooth development. Although mice may present some technical challenges, including the small size of the mouse molars, they have similar stages as humans for molar development, and can be used to monitor the effects of various biomaterials, regeneration, and remodeling. Thus, mice provide an ideal alternative model to study developmental and regenerative processes in dentistry.

Keywords: Morphogenesis, Mouse, Odontogenesis, Root canal.

INTRODUCTION

Mammals arose approximately 60 million years ago in a swift species diversification. Based on morphological and fossil evidence, the sprouting of the different mammalian arrangement is difficult to determine. According to assembled whole genome sequences, it could be confirmed that rodents are less linked to primates than carnivores. It is anticipated that animals with a bony skeleton are widely used in research and instruction. However, there are no precise and widespread figures presented on how many animals are used and/or for what purpose.

The biocompatibility of novel and/or modified materials or devices needs to be analyzed or re-analyzed. The secure use of the tested materials for humans is the main aim of this evaluation process. To evaluate the biological effects of devices and materials used in dentistry, International Organization for Standardization (ISO) standard 7405 determines test methods [1]. Animal species used for these studies comprise (in upward order of frequency): cats, primates (including monkeys and chimpanzees), dogs, farm animals (including pigs and sheep), hamsters, guinea pigs, rabbits, birds, rats, and mice.

According to the ISO 7405 [1] standard only mammals including dogs, ferrets or miniature pigs, and monkeys are suitable species to examine biocompatibility, when dental materials are in direct contact with dentine and dental pulp tissue. In the list of appropriate animals rodents are not mentioned. Consequently, when pulp capping

Received: Apr. 24, 2015: **Accepted**: June. 2, 2015

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pISSN 2321-8568

eISSN 2348-4691

materials or tooth fillings access vital dental pulp tissue highly developed animals are suggested for biocompatibility testing. Rat molar teeth were used in numerous published studies in dental related journals make it inevitable to assume that they provide an appropriate research model for these kinds of studies. It should also be considered how outcome of rat molar teeth investigations balance with results obtained in humans including bone modeling and remodeling properties, as well as bone composition and microstructure. However, some technical difficulties, such as the small size of the rat molars must be taken into consideration before pledging any research.

The complex host response, primarily responsible for disease, cannot be re-created in vitro. Thus, animal models applied in biomedical research may offer substantial advantages. The objective of the healing process in using tissue engineered bone must be cautiously taken into account, specifically, whether the priority of the study is mechanical or biologic. If the study's interest relates to biomechanics, the larger, more developed and as a result more expensive animals, may be the better choice due to approximating the size and skeletal anatomy of the human more closely. Although larger animals may provide advantages for studies that involve biomechanics, they offer little advantage looking into biological questions. Smaller, skeletally mature animals may be more helpful in examining biological queries based on their faster healing process and decreased cost, which will provide vast amount of information. Finally, the number and size of substituted materials must be considered for selecting the appropriate animal size. It should be mentioned that no single animal model will be ideal for all purposes, nor can a model be dismissed as inappropriate for all purposes, especially in dentistry. Here tooth development in the mouse was studied to provide an overview of current possibilities and limitations of this animal model in dentistry. Current review aimed to compare the suitability of mouse models at different ages in the preclinical evaluation of medical devices.

ANIMAL MODELS

Animal models help science to create new knowledge in biological sciences, including disease models for orthopedic and dentistry. Various species have been used to survey the pathogenesis of apexogenesis and to assess therapeutic modalities against the diseases, which occur spontaneously or are tentatively aroused in animals. The use of proper animal models has caused serious concerns among scientists. This may initiate from the lack of knowledge, which has not been sufficiently expanded. For instance, there was a frequent declaration between 1965 and 1977 representing that human osteoporosis is a disease of basic multicellular unit (BMU)-based remodeling and animals lacking functional amounts of BMU cannot provide good models of the human disease or its treatment. However, according to Frost et al.[2] the rat, although lacking functional BMU, can provide a practical model for human osteopenia and other skeletal problems. Thus, the proclamation that rats and mice are not proper models for study of osteoporosis originated from lack of sufficient knowledge and misjudgments[2].

Primates

Most primates have oral structures and teeth similar to those of humans, and have naturally occurring endodontic problems [3]. Some animals like monkeys and baboons are vulnerable to naturally occurring periodontal diseases [4]. Monkeys like human have a total of 32 teeth with a dental formula of incisors 2/2; cuspids 1/1; premolars 2/2; and molars 3/3. Plaque-accumulating devices are frequently placed apical to the interproximal region around selected teeth to advance plaque formation and simulate root canal abscess to use as a study model. In this part human and different animals used in dentistry research will be briefly compared.

Monkeys are extensively used in a wide range of in vivo studies such as toxicology, transplantation, dentistry, biological warfare and bio-defense, and drug testing. Investigation of the diseases like dental caries is performed on monkeys due to their comparable developmental stages to humans. There is a duplication in the number of naturally acquired *S. mutans* if the monkeys are treated with a diet similar to humans [5]. Reduction in caries results from immunization with *S. mutans* and their number also found lessened in the plaque of irus monkeys immunized against caries [6].

Among primate species, marmosets and squirrel monkeys are relatively easy to handle and are small in size. Unfortunately, the inflammatory characteristics of human periodontal diseases have not been demonstrated in these monkeys. Periodontal tissue samples obtained from these species revealed very few numbers of lymphocytes and plasma cells, which distinguish them from humans [7]. In addition, monkeys are expensive, hard to maintain, and are savage [8]. The necessity of being housed with other animals seems to be crucial for monkeys as they are considered social animals. Particularly, chimpanzees due to their appreciatively developed mental, emotional, and social prominent quality and vulnerability to suffer from living in captivity should not be used in invasive research. Consequently, high priority is placed on these species to be withdrawn from biomedical research and placed to the right wildlife refuge. Thus, monkeys are not preferred as a widely used animal model in the fields of bone regeneration, bone healing and laser applications [9].

Pigs and humans have similar oral and maxillofacial structures in terms of anatomy, physiology, and disease development. Pigs have a total of 44 teeth with a dental formula of incisors 3/3; cuspids 1/1; premolars 4/4; and molars 3/3. The Minnesota miniature pig (minipig), which has been used in biomedical research was developed more than half a century ago [10]. After 6 months of age, minipigs show plaque accumulation and bleeding following oral examination. Similar to human's histopathology there is penetration of inflammatory cells in the gingival tissue that results in progression to severe periodontal inflammation. Periodontitis in minipigs is elevated using ligatures, and in association with bacterial inoculations of *P. gingivalis*, *S. mutans*, and *A. actinomycetemcomitans* within 4-8 weeks [11]. It should be mentioned that minipigs are quite expensive and with husbandry issues.

An appropriate animal model to investigate endodontics problems is dog with a total of 42 teeth with a dental formula of incisors 3/3; cuspids 1/1; premolars 4/4; and molars 3/2. [12]. Dogs are widely used in the periodontal tissue regeneration studies and as periodontal disease models [9]. In dogs, the subgingival plaque share similar bacteria to those of human involving predominantly anaerobic gram negative cocci and rods, *P. gingivalis* and *F. nucleatum* [13, 14]. The severity of the disease has a close relation with age, and commonly ends in tooth loss. Genetic variations, rather than diet, are considered as the main factor influencing susceptibility or resistance to periodontal disease in different species [15, 16]. Furthermore, dogs are used for surgical manipulations, such as wound healing and regeneration in periodontal pockets [17].

Other researchers have introduced sheep as a new animal model. The proposed model is suitable for working out various periodontal surgical procedures [18]. Sheep are successfully used as an appropriate animal model for periodontal wound healing [19]. Goats also are used to evaluate the clinical applicability and biological response of bone fillers around oral implants [20]. Sheeps have a total of 32 teeth with a dental formula of incisors 0/3; cuspids 0/1; premolars 3/3; and molars 3/3. Despite all these information and animal species, a perfect animal model which covers every needs of periodontology does not exist, since every appliance requires a model that fills a specific need [9].

Rodent Models

Rodents including mice, rats, and hamsters, have been widely used for periodontal investigations because of their unique advantages such as known age and genetic background, low cost, small size, controllable microflora, and ease of handling and housing [21]. However, humans anatomical structures of periodontal tissues and histopathological characteristics of periodontal diseases are different from rodents [21]. For instance, keratinization of oral sulcular epithelium occur in rodents, but not in humans [22]. Periodontally involved human tissues show a complex infiltrate of lymphocytes, plasma cells, macrophages and neutrophils. In contrast, in periodontal lesions of rodents neutrophils appear to be the only infiltrating cells [21]. The divergence in the tissue reactions of rodents and humans to specific test might contribute to the possibility of some fundamental differences in host tissue responses [21, 23].

Rodents offer some special advantages to evaluate microbial and host responses to advance human periodontal studies. Rodents have only one incisor and 3 molars on each semi-mandible in comparison with humans. In all vertebrates, the

hard dental tissues together with cementum have largely similar compositions with enamel build up to 98% hydroxyapatite [24].

Some diseases are created via placement of ligatures in the gingival sulks around the molar teeth of rodents by rising biofilm agglomeration, likewise enhancing osteoclastogenesis and bone loss occurring by disrupting the gingival epithelium [25]. In other models, selected human pathogens are used to infect the animals orally to investigate the toxicity of these species in rodents [26, 27]. These approaches have also made it possible to use genetically manipulated strains to center attention on individual components of the host response, and in this manner describe their function in the disease process [28]. Lately, different researchers have used gingival tissue inoculated with chemicals, microorganisms or their products to induce periodontal disease [29-32]. A list of human diseases studied in rodent models is presented in Table 1 [33].

Rats

Rats comprise 21% of all animals used in research, which make it the second most commonly used animal species in biomedical research and testing. A somewhat deceptively low figure, but when rat and mice linked together, these two species accounts for 88% of all research animals. These animals show unique characteristics like well-defined physiologic parameters, some have spontaneous diseases useful in modeling, can be obtained with different genomes, microbial status, the easy to house and handle, inexpensive, and are adaptable to novel situations and environments. They also might carry less social concerns than the primate models. Furthermore, periodontal anatomy in the molar region of rats shows some similarities with that of humans. Thus, rats are often used in models of experimental periodontitis. Rats provide a suitable model to investigate calculus and caries, although they have limitations as a model for periodontal diseases [34]. For other implementations, rats were not commonly recommended [9]. Several strains with different characteristics are also available for experimental research such as albino Wistar rats and Sprague-Dawley rats, which are characterized by their serenity and ease of healing. Immune-suppressed and knock-out strains are also available. Rats, like mouse, have a total of 16 teeth with a dental formula of incisors 1/1; cuspids 0/0; premolars 0/0; and molars 3/3.

To evaluate the biocompatibility of medical devices used in dentistry and testing dental materials usage in pulp and dentine, preclinical molar teeth of rats can be considered as an applicable model. Studies confirmed that research applied in rat molar teeth is similar to humans, and other animal species. It is requested to amend the ISO standard 7405 to explicitly approve the use of rat molar teeth as a useful model in direct pulp capping tests. The number of currently used higher animals like monkeys, pigs or dogs for dental material testing and preclinical evaluation of biocompatibility of medical devices in dentistry can be significantly reduced if they are substituted with rats. Tests in higher developed animals should be limited to experiments clarifying challenging results.

Mice

Mouse are used to study embryology, immunology, aging, behavioral research, convulsive disorders, diabetes and obesity, infectious disease research (bacterial, fungal, parasitic, viral), and ophthalmic research. Mice are small animals, and might present technical challenges. However, advantages of using mice could be known genetic background, known age, relatively low cost and the ease of handling and housing, which make mice to be largely used in these studies. Greater concentrations of antibodies could be produced from mice monoclonal antibody than polyclonal generation in larger species such as rabbit, goat, or sheep with less effort and expense. Dental anatomy of mice, like other rodents, differs from humans. For example, the oral sulcular epithelium is keratinized in mice while it is not in humans. Mice are also extensively used in study of cardiovascular development, function, and various heart diseases [35].

Mice are available in different strains including regular, transgenic, or immune-suppressed (nude/athymic). The immune system is bypassed by breeding mice without a thymus gland in immune-suppressed strains. The body's physiological ability to distinguish between its own and another animal cell, even from the same kind, and then initiate an immune response against

foreign cells or substances are indicated in all animals in early immune function research.

Mice have 20 chromosomes in their haploid genome [36]. The haploid genome is about 3 picograms, similar to humans. Although, the gene order of the mouse and human are conserved (synteny), there are several rearrangements per chromosome. Unlike the mostly metacentric chromosomes of humans, all mouse chromosomes are acrocentric. Adult mice weigh 30-40 grams (50,000 to 70,000 grams for a young adult human) have a blood volume of 2 ml (4,800 ml for humans), and a resting heart rate of 500-700 bpm (60-80 bpm for humans). The advantages of inbred strains of mice include the fixation of genetic background and the reproducibility of that background in different laboratories and through time. For some strains, like the common C57BL/6J, the strain has been archived as frozen embryos and the stock is replaced from the frozen embryos periodically. Given the mutation rate ($1x10^{-5}$ per locus per gamete), genetic drift is low, and all mice of a given strain are essentially genetically identical. The life span of mice also varies with strain, but is typically 1.5-2 years. Known genetic background, and minimal expense for purchase and maintenance, make mice as an ideal candidate for research in mammals [37].

The nu mutation was first reported in 1966 in a closed stock of mice in a laboratory in Glasgow, Scotland. It was not until 1968, however, that it was discovered that the homozygous nude mouse also lacked a functional thymus, i.e., it was athymic. The mutation produces a hairless state, generating the name "nude." The other unique defect of nude mice is the failure of the thymus to develop normally to maturity. The thymus remains rudimentary and produces reduced numbers of mature T cells. This means the nude homozygote mice (animals with identical mutant genes at corresponding chromosome loci) do not reject allografts and often do not reject xenograft (tissue from another species). The discovery that human neoplasms (tumors) could be grown in nude mice was immediately recognized as an important research tool. Thus, the spontaneous mutation of nu among laboratory mice was a serendipitous development that led to the nude mouse becoming the first animal model of a severe immunodeficiency.

During routine lab tests on the immune system in mice another strain was discovered in 1980 [38]. The first severe combined immunodeficiency (SCID) mice were an accident of nature. At first, the SCID mouse attracted interest because it was the first known animal model for human SCID, a congenital syndrome that is usually fatal in human babies. The SCID mouse is also an excellent model for studying the relationship between immune defects and cancers of the lymph system. Dr. Bosma and his colleagues also noted that, like nude mice, the normal immune function of SCID mice could be genetically reconstituted by "seeding" with lymphocytes from bone marrow of normal mice. However, because the SCID model lacks both B and T cells, it presents much greater potential for studies of selective reconstitution of immune cell populations. Two other single-gene immunodeficient mouse models are beige and Xid mutations, which have less severely compromised immune systems than the nude and SCID models.

The C57BL/6 nude is also a general-purpose strain suitable for a wide range of studies requiring an immunodeficient research animal. The investigator can select an inbred model, in which all animals are genetically identical, or an outbred model, which has animals representing a diverse gene pool. The defined genetic characterizations of certain inbred strains of mice, such as endotoxin resistance, have made these strains of mice a useful tool in unraveling the biology of sepses and infection. In addition, inserting biomaterials subcutaneously into the back of the nude mice is also a frequently used model in bone tissue engineering [16]. The suppressed immune system also allows testing of human cells. Outbreed models, such as the Swiss nude, are more economical to produce because Swiss females have good nurturing instincts and abilities, thus producing larger litters with more robust pups. The double-mutant C.B-17-scid-beige model is deficient in B, T and NK cells, making it valuable for cancer research because one has removed another layer of immunity-the [NK] population of cells that kill tumors. Another immune deficient model, the athymic rat, has very similar mutation as the nude mouse, but because of the rat's larger size it is a better research tool for investigations requiring extensive surgery. The high reproductive efficiency of ICR/Jcl strain is a specific characteristic [45]. The gestation period for the

ICR/Jcl strain (19.5 days) is significantly shorter than the albino strain [46]. Similar embryonic stages of development to human are detected in the ICR/Jcl molar. To understand different aspect of the odontogenesis (pathology and biology) of human teeth and other calcified tissues the mouse molar can be an appropriate model [47].

USE OF MOUSE IN DENTISTRY

The Baker mouse model of periodontitis has been used to measure alveolar bone resorption caused by oral bacterial inoculums as an outcome for the clinical presentation of periodontitis in humans [39]. To assess the virulence of periodontal pathogens, specific pathogen-free female BALB/c mice were orally infected with strains of *A. actinomycetemcomitans* and/or *P. gingivalis* [40, 41]. Prior to infection, mice were given antibiotics (sulfamethoxazole and trimethoprim) in their water for 10 days to suppress the normal oral microflora. Mice were treated by oral gavages five times at 2-day intervals with one type or an admixture of bacteria resuspended in carboxymethylcellulose to establish the infection. Alveolar bone loss was detected after 10 weeks. It was speculated that *P. gingivalis* initiated experimental periodontitis, at least in part, by modifying the endogenous subgingival biofilm to acquire enhanced virulence [42]. Mice naturally develop periodontitis starting at about 9 months of age with further increases as a function of age, similar to human periodontitis. This model, however, may not reproduce all aspects of human periodontitis initiation and progression; the bacteria used are one or two of at least 150 microbial types present in any dental plaque biofilm. However, mice can be utilized to understand the host-parasite interaction [43]. Young mice also can develop periodontitis caused by their own flora, if their ability to control their indigenous bacteria is compromised by genetic defects in their phagocytes. However, the presence of antibiotics prevents the development of the disease [44].

Trinitrobenzene sulfonic acid (TNBS) or dextran sulphate sodium (DSS) are used as an alternative method for inducing inflammation of oral tissues [29, 30]. To evaluate progression of inflammatory bowel disease (IBD), these chemicals are often utilized to induce acute (1 cycle) and chronic inflammation (3–5 cycles) in the gut [48-51]. TNBS delivered rectally and DSS provided orally elicit gastrointestinal inflammation, linked with the natural microbiota of the murine gut [52-54]. DSS is an immune cell activator acts to undermine the epithelial barrier, resulting in innate immune damage to the tissues. TNBS induces a T-cell-mediated response and appears to happen to modify autologous proteins, resulting in autoimmune-like inflammatory responses [55]. In addition, these compounds unregulated ROS to create a reproducible model of IBD [48-55]. Chronic oral mucosal inflammation and alveolar bone loss results from oral delivery of DSS or TNBS for an extended period of 18 weeks [30, 56]. Systemic disease manifestations developed in mice treated biweekly with DSS in their diet, including diarrhea and colitis, and dysregulated hepatic concentrations of antioxidants in a time-dependent manner that correlated with a significant increase in alveolar bone resorption.

Systemic clinical symptoms were not detected in mice treated orally with TNBS 2 times/week [29, 30]. Oral administration of TNBS resulted in a localized action on periodontal tissues with alveolar bone loss observed in both maxilla and mandibles with progression in a time-dependent manner. In contrast, TNBS injection into gingival tissues caused a localized but severe and acute infiltration of inflammatory cells, granuloma formation, and rapid and extensive alveolar bone loss. Implementation of these inflammatory bone resorption models will enable determination of ROS contributions to inflammatory disease lesions in the oral cavity [29].

During mouse embryogenesis, the late onset of tooth development makes the mouse dentition an accessible model system for diverse types of developmental studies. Due to limited accessibility in mice, intra oral surgery approach is hardly feasible. However, periodontal regeneration and tissue engineering approaches are possible [57]. First, cells of human periodontal ligamen (PDL) were seeded in the scaffold and then subcutaneously grafted into mice. Results indicated that the gene-activated scaffold showed much better proliferation properties of human PDL cells than on the scaffold without gene-activation. Moreover, only in gene-activation scaffold the expression of the platelet-derived growth factor B (PDGF-B) and type 1 collagen appeared to be unregulated. Implanting periodontal cells

subcutaneously, bypassing intraoral surgery approach, could be a practical model for the preliminary testing approach of a newly develop material.

Transgenic mice

In recent years the significant advances in genetics and molecular biology have allowed scientist to engineer the genome of laboratory mice to add or remove genes, and even substitute chosen genes to satisfy specific research needs and protocols. The interest in immune deficient animals has been spread rapidly. To demonstrate either a gain of function (expression of a novel gene) or a lack of function (knockout of specific gene) many transgenic animals have been designed. Mouse models of many human diseases, including anti-oncogene deficiency, Gaucher's disease, retinoblastoma and others have already produced with respect to the knockout technique. Investigators' abilities to reveal the functions of specific combinations of genes and to more precisely model features of the human immune system and diseases have enhanced in consequence of the expanding ability to generate knockout animal models. An investigator can introduce an animal model in which expression (or the lack of expression) is highly anticipated by using embryonic stem (ES) cell gene deletion technology. The electroporation method is used for transformation of cultured ES cells with recombinant DNA. Unpredicted genetic interactions, incomplete deletion of targeted gene, or genome redundancy may be the source of unpredictable expression patterns in all transgenic models.

Tissue regeneration medication using stem cell is a favorable approach for regenerative medicine. A bio-root and its related periodontal tissues could be regenerated with the opportunities offered by the stem cell-mediated root regeneration and sustain the physiological function of teeth. Stem cells are characterized by two main properties. They have the potential for self-regeneration and also give rise to cells that either maintain stem cell character or give rise to differentiated cells. Regenerative treatment methods are also feasible in endodontics due to recent advances in dental pulp engineering.

Extracted third molar dental pulp tissue was the source of the first type of human dental pulp stem cells (DPSC) and characterized in comparison with bone marrow mesenchymal stem cells [58]. After DPSC seeded into dentin discs or cylinders and subcutaneously implanted into immunocompromised mice pulp-like tissues were regenerated [58, 59]. Close connection formed between the dentin and the surface, and differentiated into odontoblasts-like cells that make the dentinal tubules [58, 60]. Inside the dentin cylinder a vascularized soft connective tissue comparable to dental pulp was also detected [61]. Moreover, the amount of pulp-like tissue and dentin engendered during the organs existence is far less than the amount formed in these transplants. Another mouse model study revealed that when pulp cells were transmited into collagen gel and placed into a canal space, the shrinking hindered the pulp regeneration. The result was confirmed in an in vitro study, where the entire canal space was filled with cells/collagen gel immediately after transmitting but experienced shrinkage over time [60].

It should be noted that the theoretical aspects of laboratory biosafety have been tremendously essential to the research community. Researchers utilizing transgenic animals for disease-related study should familiarize themselves with the principles encoded in the CDC-NIH publications Guidelines for Research Involving Recombinant DNA Molecules and Biosafety in Microbiological and Biomedical Laboratories.

MOUSE TOOTH DEVELOPMENT

Tooth development is a particularly interesting model to study epithelial-mesenchymal interactions and developmental processes [62-64]. Stomodeal epithelial cells and mesenchymal cells complementary interactions in teeth bring about the different induction stages that precede morphogenesis and differentiation [65, 66]. In the course of tooth development abundant transcription factors, growth and differentiation factors, and adhesion molecules have been identified, and the dependence of tooth morphogenesis on expression of multiple genes have been revealed [67-69]. Two computational models of tooth morphogenesis have been proposed [70, 71]. The accuracy of these models differ in their

prognostication, details, realism of the cellular behaviors, and tooth formation gene networks [72].

First, the reaction-diffusion model which consists of four cell behaviors: cells can secrete and receive signaling molecules, and can divide and differentiate. A network of gene products is proposed in the model that regulate these behaviors and interactions among them [72]. A regular rectangular grid laid above three layers of mesenchymal cells including four epithelial cells compromised the base of this model. All epithelial cells in response to the local activator concentrations at an intrinsic rate secrete activators. The epithelial cells, as the result of increased level of local activator than a set threshold, differentiate permanently into non-dividing knot cells, which in turn secrete inhibitors at a ratio proportional to the local activators concentration. These inhibitors counteract activators secretion and, furthermore; enhance growth of the three-dimensional mesenchyme where diffusion and growth take place.

In another morphodynamic model, BMP2, ectodin, and FGF-4 are considered to play a role. Ectodin is an extracellular sequester of several BMPs, which in the model reduced the level of free diffusible BMP2 and BMP4. As in mouse teeth, the knots secret FGF-4 and BMP2, which facilitate mesenchyme proliferation and enhance differentiation, respectively [73, 74]. In the model, Shh is assumed to be the inhibitor, and BMP4 is the activator. Mild effects on the dynamics of models appear by these genetic differences. Considerable changes have been applied at proliferation, cell biomechanics, and growth dynamics. In contrast to the reaction-diffusion model, this model does not restrict cell position and displacement to a rectangular grid. Even when cells change their shapes this method allows realistic computations because the grid deforms and grows due to cell proliferation. The model only considers tooth development after the bud stage, because the model initially simulates flat epithelium and commences with 20 layers of mesenchymal cells covered with 19 hexagonal EC arranged in a hexagon. Each cell forms a three-dimensional moiety, which is consist of the cell itself and its surrounding extracellular space. For more insight into this model please see [75, 76].

Mice molar have characteristics which make them more appropriate than rat molars in dental research. The eruption sequence of mice molars are describe in following sections. Mice have only two kinds of teeth, as it is schematically presented in Figure 1 (A), one incisor in the front and three molars in the back which are separated by the diastema (an area with no teeth) [77]. Human dentition consists of canines and premolars, which are extra tooth types in comparison to mouse with higher levels of patterning and complexity [78].

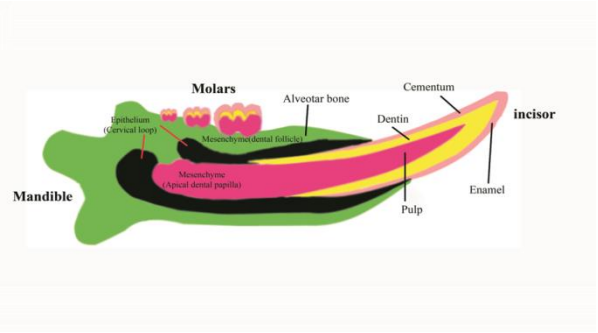

Fig 1a: Mouse dentition which contains one incisor and three molars in each quadrant.

In tooth development, morphogenetic events are initiated by the signals from the epithelium, while the first signal comes from the mesenchyme, inducing differentiation in all ectodermal organs [79]. Maturity from initiation to eruption is governed by a sequential and reciprocal signaling process by growth factors rather than straightforward one-way messages. These signaling which are needed simultaneously during critical stages of development involve all major signaling pathways, including TGF, FGF, Notch, and EGF signaling. Turecková et al [77] investigated the expression patterns of the BMP-2 and BMP-4. The msx-1and msx-2 genes of mouse embryonic upper diastema and molar regions in day 10-14, believed to regulate early tooth development using 49 series of frontal sections. At embryonic day 13 (E13) BMP-2 and BMP-4 expression was down regulated in the diastemal dental primordia during their regression [77]. The disappearance of diastemal rudiments may be related to the temporo-spatial pattern of BMPs expression. After the stage of epithelial thickening, msx-2 gene expression was not detected in the diastemal dental rudiments dissimilar to the molar origin. The msx-2 gene deficiency may affect retardation of diastemal

dental primordial growth resulting in their involution. Several recombination studies have revealed that only the very early first arch epithelium cells (occurring during the 8–11.5th E of mouse development) and ectomesenchyme (E12) have odontogenic potential [80, 81].

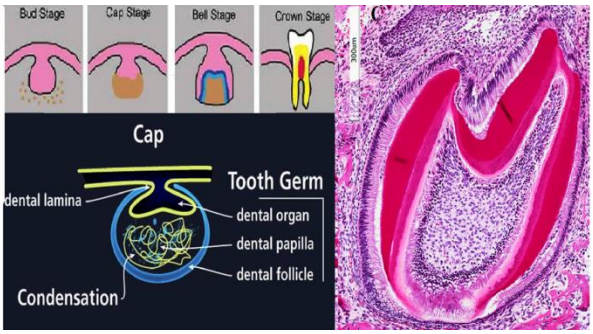

Fig 1b: Schematic representation of C57BL6 molar tooth development. Genes essential for tooth development are indicated at the developmental stage at which tooth development arrests in mutant mice. They are highlighted in yellow, blue or red, depending on their requirement, respectively, in the epithelium, mesenchyme or enamel knot. Red arrows represent the reciprocal signaling between epithelium and mesenchyme during advancing tooth development.

Initiation, morphogenesis, and differentiation are three major stages in tooth development, which are schematically presented in Figure 1 (B) [82]. In human at the end of the fifth week of pregnancy the initiation of tooth begins (the E10 of mouse development) [78, 83]. The first tooth development morphological sign is oral ectoderm thickening, which is followed by budding of the epithelium and agglomeration of the mesenchymal cells derived from neural crest near the bud. The epithelium signals stimulate the mesenchymal cells to proliferate and surround the epithelial bud. The potential of odontogenic activity during the bud stage is switched from the epithelium to ectomesenchyme. A cap-like structure is then formed from folding morphogenesis and bud development. The dental follicle or dental sac forms from cells adjacent to the dental papilla and those that lie outside the enamel organ divide and grow around the enamel organ. These three structures establish the tooth germ and give rise to the tooth and its supporting structures. At this stage, a group of agglomerated cells can be observed above the dental papilla mesenchyme inaugurating a transient signaling center. This center is the enamel knot, which soon degenerates by apoptosis [84]. During the bell or differentiation stage, the tooth crown final shape develops. At late bell stage, epithelial cells of inner enamel stretch and differentiate into ameloblasts, which are the future enamel-forming cells. The cells of the dental papilla differentiate into odontoblasts, and as they differentiate, they elongate and secrete the dentin matrix [84].

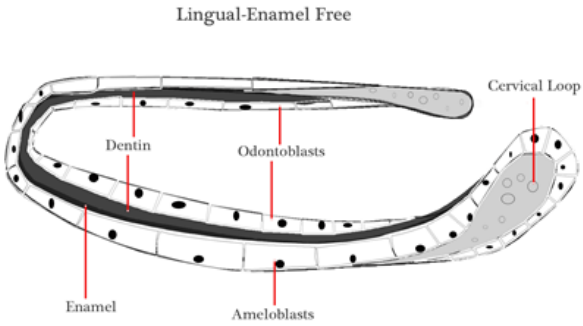

Fig 1c: Mouse incisor structure. Cytodifferentiation begins at the late bell stage when epithelial cells on the side of the incisor differentiate into ameloblasts, which secrete the enamel matrix. The stem cells in the cervical loop region support the continuous growth of rodent incisor.

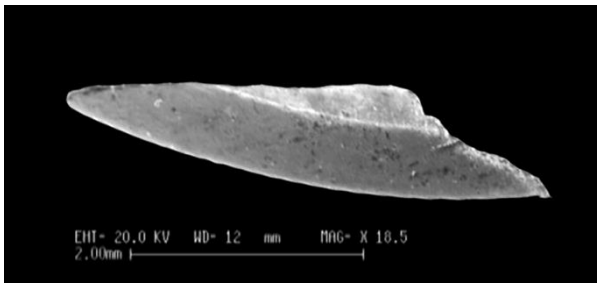

Fig 1d: Scanning electron micrograph of mice incisor (magnification=18.5 X).

Incisors are mono-cuspid teeth in contrast to the multi-cuspid molars. In rodents, only the incisors labial side is covered with enamel, while the lingual surface is covered by cementum and not enamel. Odontoblasts and dentin matrix are distributed on both sides of the incisors. A characteristic feature of rodent incisors is their continuous eruption throughout life implicating the presence of stem cells in the cervical loops at the base of the tooth [85-88]. In sagittal sections, all developmental stages from the undifferentiated precursors at the apical end to the fully differentiated cells at the incisal end can be observed. The structure of mouse incisor is presented in Figure 1 (C and D). All teeth are consist

of the same tissues, and experience similar maturation processes, regardless of shape or identity [64, 89].

Tooth development in albino mice

Briefly, after E11, Pax9 expression not only has become independent of activating epithelial signals, but also BMPs are no longer able to inhibit Pax9 expression in explants of the mandibular arch mesenchyme [90, 91]. There is no sign of the dental lamina differentiation. First dental structure appears prior to E12 [46], and E12 is when the earliest indication of odontogenesis appears. Several mitotic figures revealed form oral ectoderm and underlying mesoderm. All four major morphogen families (BMPs, Shh, Wnt, and FGFs), as well as other genes linked to signaling including p21, Msx2, and Lef1, whose expression lasts during the bud stage [47, 92; 93].

At E13, the epithelium and the mesenchyme form a bud which starts to condense [91]. Due to cell division, a significant thickening and elongation of dental lamina occurs [46]. Around E14, the length of the dental lamina increases as a result of anterior-posterior growth, and the dental papilla becomes visible [46; 91]. At this time, both the upper and lower first molar enamel organs make their appearance. A club-shaped tooth bud forms from rapid cellular proliferation of the dental lamina [46]. The length of dental lamina continues its growth and outer enamel epithelium proliferation seems to stop on E15 [72]. As the first sign of cap stage, invagination emerges on the deep surfaces of the first molar tooth buds [46, 91]. Another knot forms buccally from the first one and over time, they end up in the tip of two bumps, and a valley forms in the epithelium between these bumps as a result of proliferation which deepens the epithelium into the underlying mesenchyme [72]. A new knot forms by E16. The posterior forms the first, and soon afterwards a fourth forms lingually from it [46]. The outer enamel epithelium stops proliferating and its cells flatten. Meanwhile, the growth of cervical loops continuously changes from growing laterally to mainly growing downward extending from anterior to posterior direction, which give rise, in mouse, to the tooth root [72]. At this time, second molar tooth buds could be detected [46].

The growth of the first molar enamel organ at E17 is accentuated, mainly in its cervical region. An epithelial island which will participate in root formation is also evident for the first time [94]. The second molar enamel organ is in the cap stage and is similar in emergence to the first molars at E15 [46]. The bell stage of enamel organs of the first molars begin during E18 period. The thickness of outer enamel epithelium reduction and the slow increase in the inner enamel epithelium height also occurs. The second molars enamel organ at E18 and the first molars at E16 are comparable [46]. At E19 and E20, the first molar's crown pattern is almost completed and certain cells of the inner enamel epithelium differentiate to ameloblasts. [46]. This cyto-differentiation of ameloblasts begins at the high point corresponding to growth centers on the cusps. On E20, predentin formation begins by the differentiation of dental papilla cells to odontoblasts. For second molars this day is comparable to first molars at E18.

The first molar's crown pattern is completed on P1-P2. On the surface of cusps, enamel matrix is formed from ameloblasts and its differentiation continues down the slopes. The rhythmic pattern of amelogenesis and dentinogenesis in the first molars is constructed. On P2 the dentinogenesis begins in the tooth germs of the second molars [46]. On P3-P4 the first molar crown is covered with a layer of dentin as a result of rapid progress of dentinogenesis and amelogenesis, and calcification of predentin and formation of enamel matrix begins. The second molars enamel organs crown pattern is also complete on P3. By P10, the first molar's enamel matrix thickness is laid down, crown pulp chamber width decreases, cusps tips enamel maturates, root forms, and eruption begins. During P16 to P20, eruption of the first molars are continually completed. For the second molars the mentioned phenomena begin between P11-P12, and are noticeably promoted by P15 and erupt between P18 and P19. On P24 and P25 roots of the first and second molars reach their mature length, respectively. The third molars erupt between P28 and P29, and functional closure will reach by P36.

A summary of development and morphological features of albino mouse molars, and ICR/Jcl first mandibular molar is also presented in Table 2 and Table 3, respectively [46, 69]. The mammalians tooth replacement mechanisms are still uncovered, mainly because the most used model specie, the mouse, lacks the ability to replace

its teeth [24]. Mammalian individual molars develop from the dental lamina distal extension, and do not have replacement teeth. As other teeth of the primary dentition, molars develop consecutively. Thus, in mice and humans the third molar (the wisdom tooth) is the last to develop. Whereas the first mouse molar develops quite normally when cultured in vitro, the second and third molars are frequently delayed or missing in cultured conditions [24]. However, Kavanagh et al. [95] were able to rescue the development of distal teeth by dissecting the posterior extension of dental lamina and culturing it separately from the first molar. This was interpreted to be due to inter-molar inhibition, where the size and number of distal molars depend on the balance between mesenchymal activators that promote enamel knot induction, and inhibitors that are expressed in the previously initiated molars.

That absence of third molars is common in an inbred strain of mice (CBA), while another inbred strain (C57BL/6) seems to be entirely free of this anomaly. C57BL/6 mouse have larger third molars compared with CBA mice. In addition to the fact that the crowns of C57BL/6 mice teeth are larger, the roots are much better developed and separated from each other. In CBA mice the roots are usually fused with each other, so that, on superficial inspection, there seems to be a single root only, as is in a human incisor. The absence of third molar occurs in 9-17 % of the mice of the CBA pure line, but has not been encountered in the C57BL/6 strain. While C57BL/6 has fairly large third molars, those of CBA are small and very variable in size. The smallest members of the series tend to be absent altogether. The mean size of the third molars is influenced by the 'vigour' of the mother. Other things being equal, young born of inbred (CBA or C57BL/6) mothers have smaller third molars than young born of hybrid mothers.

The mice molars, like the rat, during development can be used to examine alterations in response to mechanical, hormonal or metabolic influences. The incredible deposition of cellular cementum on the mature molars root tips presents excellent possibilities to those interested in studying hypercementosis [46]. A marvelous and accessible educational material source for tooth development is the mouse molar. This is mainly because of the similarities it shares with those of human teeth, like the manner of growth, calcification, eruption, and also there is no difficulty with decalcificataion.

In response to mastication and orthodontic tooth movement periodontal ligament (PDL) and alveolar bone are exposed to physical forces in vivo and are involved in remodeling of the periodontal and gingival connective tissue, as well as tooth eruption [96]. There are certainly discrepancies between rodents and humans, mainly due to differences in the PDL between the molars and the continuously erupting incisors. Rodent incisors have no roots and are continually erupting, which might be related to the derivative of the dental follicle, the PDL [97, 98]. Adult epithelial stem cells which are important for this eruption are stored in the cervical loops [99]. The presence of periostin in the periosteum and the PDL ECM of the adult mouse suggests that periostin may be involved in regulating adhesion and differentiation of osteoblasts, and in responses to mechanical forces on the teeth [100].

CONCLUSION

To provide reliable data concerning gene and systemic effect on pathology of pulp and pulp tissue reaction after direct pulp capping and related queries in dentistry, mice molar teeth might be a practical study model. Thus, the use of mice may significantly reduce the number of currently used higher animals in research. However, mice have some technical challenges, like the small size of molars. Our results showed mice have a similar scenario for molar development as humans, and can be used to monitor the effect of biomaterials, gene interactions and systemic loading of drugs on physiological and pathological development, regeneration and remodeling. Thus, mice provide a suitable alternative to study developmental and regenerative issues in dentistry.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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How to cite this article:

Saghiri MA, Orangi J, Asatourian A, Sheibani N. Validity and Variability of Animal Models Used in Dentistry. Adv Hum Biol. 2015;5(2):1-16.