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REVIEW ARTICLE

EXPERIMENTAL ANIMAL MODEL DEVELOPMENT

Utpal S. Patel* and Rohin N. Patel

Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari - 396 450

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***Corresponding author:**
Utpal S. Patel

ABSTRACT

Human or non-human species are used as an animal model in biomedical research which is helpful in determining biological process or disease found in humans and animals. In order to create treatments and prevention measures, animal models are crucial for biomedical research. Knock out mouse, naked mice, humanized mice, Sprague-Dawley rats, Wistar rats, zebrafish, non-human primates *etc.* can be used in animal model development. There are several uses of animal models such as to understand biological process, for new studies, research work and are used in preventive and curative aspects like development of vaccine and antibiotics. Several ethical guidelines should be followed during use of animal models. Animal models should be selected according to type of study. Experimental animal models can be developed for diseases such as diabetes, ketosis, atherosclerosis, cancer, wound healing, covid 19 *etc*. Humans can be the ultimate animal model for experimental study. Organ on a chip model is often referred to as micro-physiological systems or organoids; these advanced miniaturized systems are made to resemble human organs in terms of both shape and function. New approaches like genetic engineering, artificial intelligence, organ on chips, etc. are being developed as a result of reducing and replacing the number of animals employed in experimental protocols. Recently, scientists have started reconstructing germ-free mice and rats with microbiota derived from human faecal samples, going beyond the ideas of gnotobiology. For the future perspective, microchip models concentrate on discrete organs or certain activities, whereas animal models enable the study of intricate relationships within an entire organism.

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INTRODUCTION

Human or non-human species are used as an animal model in biomedical research which is helpful in determining biological process or disease found in humans and animals. We could learn much about ourself by studying animal models. In *in vivo* study, use of animal model is unquestionable in terms of implementation in biomedical research. Rodent, rabbit, non-human primate, dog and fish are some of the eminent animal models (Mukherjee *et al.,* 2022).

Uses of Animal Model

- To understand biological process.
- For new studies and research work.
- Used in preventive and curative aspects like development of vaccine and antibiotics.

ETHICAL GUIDELINES

Sited below are ethical guideline to be followed while using animals in dental research (Richmond, 2002).

- Animal studies only to be used to enhance human or animal as well as to increase the data for study
- Use of minimum number of animal models
- Ethical treatment of animals
- Painful condition should be performed under anaesthesia
- Animal with poor prognosis should be euthenized.
- Performer must be technically expert
- Whenever feasible, the number of animals needed can be decreased by using in vitro techniques and ex vivo animal models.
- The most significant rules to follow while undertaking research on experimental animal models are the notion of three Rs. Replacement, reduction, and refinement that Russell and Burch introduced. When experiment animals are used for research, three R should be followed and animal should only be used when absolutely required. The number of animals used for study should be maintained to a minimum and the animal suffering throughout the study should be minimised.
- Adittionally, 4th R is included by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in that is "rehabilitation" of animals used in experimentation.

POINTS FOR SELECTING RIGHT ANIMAL MODEL

There are several points by which animal model can be selected (Davidson *et al*., 1987).

Two Broad Classes of Models: Those based on analogy and homology.

Four Main Categories of Animal Models

1) Artificial or experimental models that make an effort to replicate the original species conditions, 2) Natural or spontaneous models that are acknowledged to resemble certain conditions in the actual species, 3) The typically opposites of a disease model, that are negative or nonreactive models. 4) Animal diseases that have no known human or animal counterparts are known as orphan models.

Bases of the animal model selection

1) Suitability of an analogy, 2) Information transferability, 3) Organism's genetic homogeneity, when appropriate, 4) Basic knowledge about biological properties, 5) Price and accessibility, 6) Generalizability of the finding, 7) Simplicity and flexibility in manipulating experiments, 8) Environmental consiquences, and 9) Ethical outcomes.

COMMONLY USED ANIMALS IN EXPERIMENTAL ANIMAL MODEL

Knockout Mouse: A mouse in which a gene is "knocked out", by substituting or interfering with an artificial DNA fragment.

Naked Mice/ Nude Mice: A naked mouse is a strain of laboratory mouse that has a genetic abnormality that results in a thymus that is either nonexistent or degraded, which inhibits the immune system since there are much fewer T cells. The name "nude mouse" comes from its phenotype, or primary external appearance, which is the absence of body hair. Because it mounts no rejection response, the naked mouse is useful for study because it can accept a wide variety of tissue and tumour transplants. These xenografts are frequently employed in studies to test novel approaches to tumour imaging and treatment. A disruption of the FOXN1 gene is the genetic basis of the naked mouse mutation (Fogh, 2014).

Nomenclature: When the altered gene was found to be a mutation in the HNF-3/forkhead homolog 11 gene, their original description of nu was changed to *Hfh11nu*. The nomenclature was changed to Foxn1nu in 2000 after it was discovered that the mutation-causing gene belonged to the Fox gene family.

History and significance: First discovered at Ruchill Hospital's in Brownlee virology laboratory in Glasgow 1962 by Dr. Norman R. Grist. Nude mice are unable to produce mature T cells because they do not have a thymus. As a result, they cannot mount a variety of adaptive immune responses. Furthermore, naked mice cannot reject xenografts (grafts of tissue from another species) or allografts (grafts of tissue from other animals) because they lack functional T cells.

Life span: The life span is normally 6 months to 1 year. In controlled environments and with antibiotic treatments, they can live upto18 months or 2 years.

Humanized Mice: Animal models are helpful in investigation of pathogenesis and therapies of disease in humans. Mice have played key role in numerous fields of biomedical research as a flexible and conventional small animal model. Diverse genetic information encouraged the use of mice as model systems. However, there is major differences between mice and humans. Although they may be preferred in this regard, laboratory animals that are more closely related to humans, such as non-human primates, have drawbacks of their own, including high expense and difficulty of access. In this case, humanised mice that have been engrafted with functional human cells or tissues offer the special benefit of enabling research on the growth and operation of human cells and tissues in the *in vivo* setting of a tiny animal. Different types of humanised mice have been created by engrafting human cells or tissues into mice, such as immune system components, hepatocytes, uterine endometrium, and brain cells. The most well researched of these are humanised immune system mice, which are engrafted with functional human immune system components. Consequently, when the word "humanised mouse" is used without more explanation, it frequently refers to a mouse with a humanised immune system (Fujiwara, 2018).

Sprague-Dawley (Sd) Rat: It is an outbred rat, generated first time by Robert S. Dawley in the 1920s by breeding Wistar rats to hybrids of laboratory derived and wild stocks. Charles River Laboratories (CRL), In 1950 developed a new line with better microbial status by caesarian derivation (White and Cham, 1998). Animal models of human diseases like diabetes, obesity, cancer, and cardiovascular disorders are frequently created using Sprague-Dawley outbreds.

Wistar Rat: It is an outbred of albino rat which is developed at the Wistar Institute in 1906. Use in biomedical research. Notably, during a period when the house mouse (*Mus musculus*) was the most common model organism used in laboratories, the first rat was created. Wistars served as the model for the Sprague Dawley and Long-Evans. More energetic than Sprague Dawleys. For example, other well-known stocks derived from Wistars include the Lewis and the spontaneously hypertensive rat (Eren *et al*., 2020).

Zebrafish: Zebrafish can be used to examine a number of human metabolic disorders, including nonalcoholic fatty liver disease, type 2 diabetes mellitus, dyslipidaemia, and other hepatic diseases, because they have all the major organs involved in metabolism (Nishio *et al*., 2012). It has 70% genetic homology with humans (Howe *et al.,* 2013), it can mimic human health and disease, while its small size and fast development help in experiments on a larger and quicker scale (Martin *et al.,* 2019; Teixido *et al.,* 2019*)*. Several transgenic models of cancer, such as melanoma, leukaemia, pancreatic cancer, and hepatocellular carcinoma, have been created using zebrafish. (Liu and Leach, 2011; Ceol *et al.,* 2011). The zebrafish has been utilised as a model for human myocardial infarction in cardiovascular research. After around two months of damage, the zebrafish heart fully regenerates without leaving any scars (Chablais *et al.,* 2011).

Non-Human Primate Model: Non-Human Primates (NHPs) are employed in biomedical research because of similarity with humans in terms of physiology, neuroanatomy, reproduction, development, cognition, and social complexity. However, it is precisely because of these similarities that the use of NHPs in biomedical research is carefully evaluated (Phillips *et al*., 2014). In comparison to rodent models all primate models show a more similarity to human-like period of development (Walters *et al*., 1987). Rhesus macaques *(Macaca mulatta*), Cynomolgus macaques (*Macaca fascicularis*), African green monkeys (*Chlorocebus sabaeus*), baboons (*Papio hamadryas*), and common marmosets (*Callithrix jacchus*) were among the NHPs employed in COVID-19.

EXPERIMENTAL ANIMAL MODEL DEVELOPMENT

Diabetes: Experimental diabetes mellitus is usually created in animals because animal models are helpful for understanding the biology of the disease (Roep and Atkinson, 2004; Arndt *et al.,* 2013). Some studies are also done in larger animals. Three categories of experimental animals are used to study diabetes mellitus such as genetically diabetic animals, miscellaneous models, and additional model dependent on how experimental diabetes mellitus is induced (Kumar *et al*., 2012). Diabetes can be induced either by using chemical agents or spontaneous methods (Chatzigeorgiou *et al*., 2009). There are two main ways to create animal models such as genetic modification or illness induction (*i.e.,* using certain medications). Both are important because they make it possible to analyse specific disease-related systems and are crucial for comprehending the disease pathogenesis, development, and extrapolation to people.

Ketosis: Blood sugar levels are lowered by ketogenic diets (KDs), which are high in fat and low in carbohydrates. The goal is to develop a low-carb diet that, in the absence of large levels of hydrogenated fat tha induces ketosis. Mice were fed a ketogenic (KD) diet for three months. Mice that follow a ketogenic diet experience mild ketosis but no changes in body weight. It reduces beta amyloid levels in the

brain, but not congophilic plaques. A ketogenic diet can induce more ketosis (Brownlow, 2013).

Atherosclerosis: Lipid buildup in the arterial vessel wall is the hallmark of atherosclerosis, a degenerative inflammatory disease that begins early in life. Accumulation of atherosclerotic plaques cause narrowing of the arterial lumen. Atherosclerotic plaques usually remain stable for long time but can become unstable ubruptly, breaks and form thrombus. It will lead to an increased risk of acute cardiovascular diseasse such as myocardial infarction. To better understand the molecular mechanisms underlying the development and evolution of atherosclerotic plaque, as well as the occurrence of plaque rupture and its related cardiovascular events, animal models of atherosclerosis are crucial. Additionally, using animal models makes it possible to evaluate new pharmaceutical therapies that may stop or delay the development of atherosclerosis. Animal models of atherosclerosis are often predicated on accelerated plaque formation caused by: (1) a diet high in cholesterol or Western-style foods; (2) gene alteration related to cholesterol metabolism; and (3) the introduction of additional risk factors for atherosclerosis, like diabetes (Veseli *et al*., 2017).

Cancer: Immunocompetent or immunocompromised mice transplanted subcutaneously or orthotopically with syngeneic and xenografted tumours are the most commonly used models for oncology research (House *et al.,* 2014); their usage is primarily supported by their low cost and ease of creation. The malignant environment in an oncological patient, is much more complicated in a therapeutic setting. DNA construct microinjection, retroviral infection, and the "gene-targeted transgene" method are the three main ways that tumour suppressor genes are downregulated or oncogenes are expressed preferentially in transgenic mice that is another type of model for cancer research used in preclinical settings.

Wound Healing: Over several decades, models of experimental wound healing have been created in an effort to test novel treatment approaches and comprehend the tissue restoration process. These models often fall into two categories such as animal (*in vivo* or preclinical) models and *in vitro* models, each of which has benefits and drawbacks (Stephens *et al.,* 2013). *In vivo* models entail injuring a lab animal and tracking the healing of the wound over time. The wound environment may also be altered physically, chemically, or biologically (Stephens *et al.,* 2013; Nauta *et al.,* 2013). Because they provide a realistic depiction of the wound environment, encompassing different cell types, environmental cues, and paracrine interactions, in vivo models continue to be the most predictive models for researching wound healing (Wong *et al*., 2011). The model that is selected should take into account features like the lesion accurate reproducibility, the potential for multiple investigations, the capacity to obtain multiple biopsy samples, compatibility with animal facilities, ease of handling, and the amount of time needed to produce useful results (Gottrup *et al.,* 2000). A model that accurately depicts some features of human physiology without requiring human subjects for research is ideal (Geer *et al*., 2004). Because small animals typically heal more quickly than humans do, experiments on them typically last days rather than weeks or months (Shrimanker *et al*., 2013). Rats and mice are the most commonly utilised species. Even though the structure and physiology of rodent and human skin have been shown to differ, investigations on wound healing that take these distinctions into consideration can yield important translational data (Nauta *et al*., 2013).

COVID-19 (SARS-CoV-2) Animal Model: Covid-19 illness induction in a non-human primate model, genetic alteration using humanised and transgenic mouse models, models based on SARS-CoV-2 strains that have acclimated to mice, Syrian hamster models and humanised mouse models with engrafted human tissues or cells. Research on the mechanisms of infection, transmission, and immune responses has aided in the development of COVID-19 vaccines and treatments during the pandemic (Muñoz-Fontela *et al.,* 2020).

HUMANS: THE ULTIMATE ANIMAL MODEL AND ORGAN ON A CHIP MODEL

Humans: The Ultimate Animal Model

Although people are the best animal models for human diseases, their use is limited, especially when it comes to researching hereditary illnesses and creating treatments. In addition to being creative in creating new methods of studying humans, we must investigate how to maximise the use of both human and non-human models in comprehending genetic disorders and creating treatments. Due to their prevalence, inherited neuropathies provide a perfect model for researching inherited disorders (Reilly and Rossor, 2020).

Organ on a chip model: Often referred to as micro-physiological systems or organoids, these advanced miniaturised systems are made to resemble human organs in terms of both shape and function. To mimic the physiological circumstances of particular organs, such as the heart, lung, liver, kidney, and vagina, these devices use microfluidic channels, living cells, and other biological elements (Moon *et al.,* 2023).

Advantages

- Precision medicine relevance to humans and animals
- Minimum animal testing
- High- screening turnout

CONCLUSION

In order to create treatments and prevention measures, animal models are crucial for biomedical research. CPCSEA (Committee for Purpose of Control and Supervision of Experiments and Animals), AAALAC (Association for Assessment and Accreditation of Laboratory Animals), OECD (Organisation for Economic Cooperation and Development), and ICH (International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human use) all create different guidelines and protocols for the use of animals. For experimental study, a variety of animal models, such as vertebrates and invertebrates, are used to mimic human and animal diseases. Significant progress has been made in rodents, non-human primates, dogs, pigs, and even invertebrates like zebrafish and nematodes in addressing global priority diseases as COVID-19, cancer, diabetes, obesity, and Parkinson's disease. New approaches like genetic engineering, artificial intelligence, organ on chips, etc. are being developed as a result of reducing and replacing the number of animals employed in experimental protocols.

FUTURE PROSPECTS

With the use of numerous models to guarantee data robustness and new genetic and metagenomic technologies to create and improve "humanised models," animal modelling will increasingly shift towards models that most closely resemble human situations. Transgenic animals that express human genes are examples of humanised models that are becoming more popular. The introduction of the gene encoding the human major histocompatibility locus, HLA-B27, into rats is a well-known example (Taurog *et al.,* 1999). The idea of producing human "organs" in mice has also spread to other systems, like the liver, where research on drug metabolism and viral hepatitis is greatly benefiting from the use of humanised animals. Recently, scientists have started reconstructing germ-free mice and rats with microbiota derived from human faecal samples, going beyond the ideas of gnotobiology (Licht *et al.,* 2007; Turnbaugh *et al*., 2009). Microchip models concentrate on discrete organs or certain activities, whereas animal models enable the study of intricate relationships within an entire organism. It is appropriate for the focused research.

Conflict of interest: The authors declare that there is no conflict of interest.

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