Review Article

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Research Models in Biomedical Sciences: Advantages and Limitations

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ABSTRACT

Research models are routinely used in the biomedical field when we are talking about assays. All these models have advantages and limitations and should be chosen carefully, to avoid, mainly, mistakes in ethical practices. However, it is a great challenge for researchers picking and classifying their experimental models due to the lack of clear literature definition or guidelines. Here, we pioneer describe the overall definition of the main research models and give a gamut of examples from the biomedical field. This review also provides recommendations for aligning experimental practices to meet the necessity of assessing an international research model classification.

KEYWORDS: *Ex situ*; *Ex vivo*; *In silico*; *In situ*; *In vitro*; *In vivo*

ABBREVIATIONS: AIDS: Acquired Immunodeficiency Disease Syndrome; CAM: Chick Chorioallantoic Membrane; CCLE: Cancer Cell Line Encyclopedia; DNA: Deoxyribonucleic Acid; ELISA: Enzyme-Linked Immunosorbent Assay; FISH: Fluorescent *In Situ* Hybridization; HIV: Human Immunodeficiency Virus; iPCR: Immune Polymerase Chain Reaction; ISCT: *In Silico* Clinical Trial; MCL: Mantle Cell Lymphoma; OECD: Organization for Economic Co-operation and Development; PCOP: Porcine Cornea Opacity Permeability; PCR: Polymerase Chain Reaction; SPR: Surface Plasmon Resonance

INTRODUCTION

Latin remains a mainstay in academic literature, particularly in natural and medical sciences [1,2]. Indeed, a plethora of scientific expressions exist that are solely used in Latin [3], e.g. the experimental models *in vitro*, and *in vivo* [4,5], and *in silico* [6,7]. Whilst it is key to select the appropriate research model for a given hypothesis to avoid waste of time and resources [8], choosing a suitable model that is capable of meeting the study objectives, is often complicated by unclear or entirely lacking literature definition of the various models; often, further confusion is added due to semantic differences between languages [9]. This review provides an outline of the most used research models, i.e. *in silico*, *in vitro*, *ex vivo*, *in situ*, *ex situ*, and *in vivo* (Figure 1) and an in-depth discussion about the advantages and limitations of each model. Furthermore, examples of different technologies applied in biomedical sciences (e.g. drug discovery) surrounding the research models of sciences will be explored, aiming to adopt clear and unambiguous definitions within the employed models.

MAIN RESEARCH MODELS

In Silico

In silico models (i.e. performed on computer) always require digital technology to simulate a specific process (e.g. pharmacologic, physiologic, etc.). Originally, silico referred to the chemical element silicon (Si) or silicium, the main compound of

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computer circuits [10]. In fact, the scientific expression "*in silico*" was first described in 1989 for experiments carried out entirely on a computer [11]. Nevertheless, some authors still consider in silico studies as a virtual extension of *in vitro* and *in vivo* experiments [12,13]. *In silico* approaches are represented by techniques that use software to analyze data and often involve computational models or simulations based on existing information of closely related phenomena. The output can then be used to make predictions and suggest hypotheses as a basis for *in vitro*, *ex vivo*, and *in vivo* models [14].

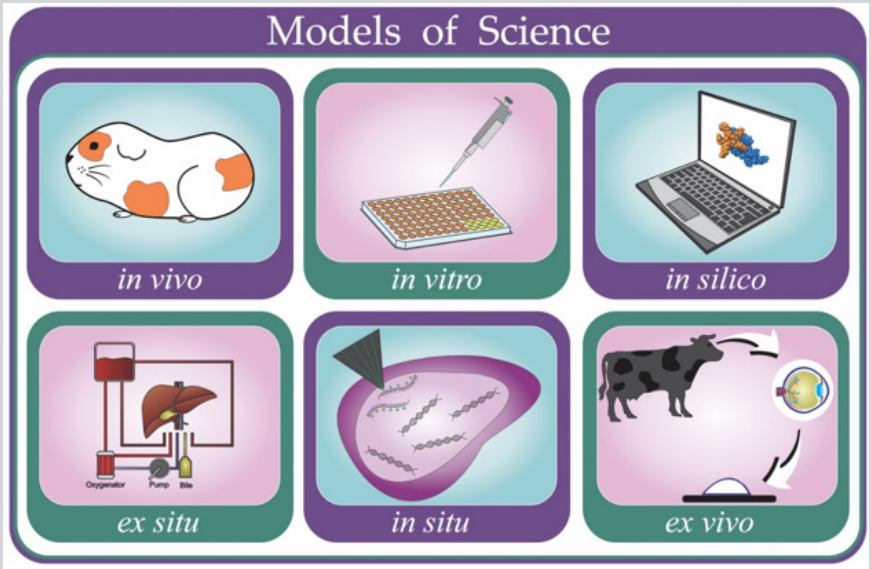

Figure 1: Research models in the biomedical sciences. *In vivo* - represented by a guinea pig; *in vitro*-represented by the 96-well plate; *in silico* - represented by a protein model; *ex situ* - represented by a perfusion system for organ preservation; *in situ* - represented by fluorescent *in situ* hybridization (FISH); and *ex vivo* - represented by ocular tests in bovine corneas.

The greatest advantage of *in silico* methods is that they are usually faster and cheaper than classical tests, whilst also reducing the number of animals to *in vivo* assays [15]. Nevertheless, there are several pitfalls that are commonly encountered when employing *in silico* assays. Mainly, *in silico* studies demand rigorous validation, including experimental verification during model development [16]. Nevertheless, researchers should not be concerned about using *in silico* approaches, which boost medical and pharmaceutical innovation, provide an affordable cost and allow huge business opportunities [17]. Thus, it is not surprising that the usage of *in silico* models has greatly increased in the last years, as demonstrated by pharmacokinetic, oncologic and drug discovery studies [18,19]. Indeed, following the development of ever improving bioinformatic tools, there was a greatly increase in the arsenal of *in silico* methodologies, which have already resulted in significantly improved prediction and elucidation of the dynamics surrounding complex biological phenomena [20,21].

Molecular docking, which explores protein-protein or protein-small molecule binding, is one of the preferred *in silico* methodologies used, especially in drug discovery [22]. Since its discovery in the early 1980s [23], more than 60 different docking tools and programs have been developed for both academy and companies [24]. Other *in silico* methods that are routinely used in research laboratories include molecular modelling (a technique used to model or mimic the structure of molecules) and protein sequencing and its alignment (methods used to evaluate identities and similarities in the amino acid sequence of proteins) [25-28].

In silico toxicity prediction is also being employed to great success [29]. The computational methods employed to predict the toxicity profile of drugs has stood out to design new medicines once as *in vitro* and *in vivo* methods are often limited by ethics committees, time-consuming, and require a lot of resources [30]. So far, more than 15 sources of *in silico* toxicity model data are available, including ToxCast, Tox21, and ToxBank [30]. Similarly, *in silico* bioprospecting is being explored for searching new molecules for different targets and applications [31]. Detailed *in silico* methods to design biotherapeutics (e.g. antibodies and vaccines) have been discussed in detail elsewhere [32].

Recently, the *in silico* clinical trial (ISCT) came to the light. This approach refers to the development of patient-specific models to form virtual cohorts for testing the safety and/or efficacy of new drugs and of new medical devices. Clinical trials involving new drugs are frequently classified into four phases (I-IV). Phase I recruits about 20 to 100 volunteers and aims to test safety and dose scalation. The use of ISCT can predict the optimal dosage. In Phase II trials, which recruit about 100 to 300 volunteers, ISCT could be used to find representative virtual patients that show adverse effects [33]. In the most expensive Phase III, conducted with 300 to 3,000 patients, ISCT can assess the efficacy in terms of reduced side effects, predict ineffectiveness, and reduce the number of enrolled patients. Thus, ISCT can meaningfully lower the costs related to Phase III clinical trial [33,34].

In Vitro

In vitro (i.e. within the glass) refers to a model in which the assay occurs outside of a living organism and in a controlled environment. In addition, *in vitro* techniques are very valuable for ethical (does not require animals) and economic reasons [35]. Although the number of *in vitro* assays could be uncountable, few examples frequently used will be explored in this section.

All analytical techniques are classified as *in vitro* techniques such as chromatographic and spectrometric techniques,

although they can be used to evaluate samples from *ex vivo* and *in vivo* studies. Chromatography is a century-old technique [36] used to separate and quantify complex mixtures of molecules according to their properties, which can be classified in different types (e.g. liquid chromatography, gas chromatography, ion-exchange chromatography, and affinity chromatography) [37-39]. Moreover, chromatography can be regularly associated with mass spectrometry (MS) analyzes, which is a powerful analytical technique for identifying, quantifying, and exploring molecular structures [40]. Both techniques can be employed to different compounds, including volatile components, lipids, organic molecules, and proteins [41,42]. Another method used to separate proteins according to their Physico-chemical characteristics (charge and size) is electrophoresis [43,44]. Electrophoresis can be performed in a gel [45] or on a filter paper [46], besides been used combined with mass spectrometry to identify protein sequence and mass (i.e. in-gel digestion) [47].

There are also other important *in vitro* methods broadly used for studying structure and biochemical characteristics of molecules, such as circular dichroism [48], protein quantification (e.g. Bradford and Lowry methods) [49,50], crystallography [51], as well as enzymatic assays, both colorimetric and turbidimetric, such as assays employed for phosphodiesterase [52,53] and hyaluronidase enzymes [54,55], respectively.

Other groundbreaking *in vitro* analytical test exhaustively used for research purposes, diagnostics, pharmacokinetic studies (i.e. drug monitoring) and for evaluating quality control of products is the enzyme-linked immunosorbent assay (ELISA), which employs enzyme-mediated color change and respective absorbance to quantify the presence of antigen-antibody mechanism [56]. ELISA method is globally employed because it is cheap, simple, specific, and quick to execute [56]. In connection with antigen-antibody binding, surface plasmon resonance (SPR) is also used to evaluate affinity of immunocomplexes [57].

Most of molecular biology assays are based on *in vitro* experiments, including the prominent polymerase chain reaction (PCR), which allows amplification of specific DNA segments through repetitive cycles of denaturation, hybridization, and polymerase extension [58,59]. In the recent years, the immune-PCR (iPCR) has frequently been employed. The iPCR method provides classical PCR sensitivity to objects traditionally detected by ELISA [60]. Phage display, an elegant *in vitro* technology in which a bacteriophage (i.e. a bacteria and archaea infecting virus) is used to evolve peptides and antibodies has been considered a robust technology for the discovery of therapeutic human antibodies against several diseases (e.g. autoimmune diseases and cancer) [59,61-63]. Still in molecular biology subject, microarray is an *in vitro* renowned technique applied for characterizing protein-protein, protein-nucleic acid, protein-small molecule, and antibody-antigen interactions. The technique has been used in both basic research (e.g. omics analysis) [64] and clinical applications (e.g. tumor markers) [65,66].

Although experiments conducted with living cells removed from humans or experimental animals should be considered an *ex vivo* method, cultures of lineage cells are better defined as *in vitro* approaches. Since 1912, when Carrel and his coworkers developed the first cell line from chicken embryo heart, cell lines have been extensively explored. However, their popularity increased in 1951, after the discovery of the immortalized HeLa cells, obtained from an adenocarcinoma of a patient named Henrietta Lacks [67]. Recently, cell lines, in special human cell lines, are generally used as a source of cells for high-throughput *in vitro* screening assays [68]. For instance, 1457 cancer human cell lines have been explored to predict drug sensitivity, which was defined as a Cancer Cell Line Encyclopedia (CCLE) [69,70]. Nevertheless, the actual number of generated cell lines is not reported and hard to estimate [71].

Similarly, there are other not obvious assays employing cells and organs described as *in vitro* approaches. The *in vitro* chicken enucleated eye test can detect the degree of eye irritation of certain compounds within six hours [72], which is considered an alternative to the *in vivo* Draize eye test method performed in rabbits [73]. Recently, an interesting new concept of *in vitro* assay emerged, named organs-in-a-chip, which describes a cell culture-based model system in which cells of different kinds are placed on small structures (chips). These chip-cells combinations can mimic the role of an organ or tissue, creating the organ-like structures. The organ-like system can be used for different applications such as drug toxicity screening [74], and it is expected that the technique could revolutionize early clinical trials in human patients by clinical-trials-on-chips [75].

Nevertheless, there is a continuous conflict between *in vitro* and *ex vivo* models [76]. Indeed, some authors use *in vitro* to refer to experiments performed within live cells (not cell lines) outside the organism, which should be best classified as an *ex vivo* approach. In any event, *in vitro* models undoubtedly provided an inexpensive and high-throughput alternative to *in vivo* research strategies. However, although *in vitro* models are fruitfully used in biological fields, extrapolation of the observed effects to animal models and humans can be considered weak and may lead to a misinterpretation of the data [77,78]. Indeed, researches that intend to use *in vitro* tests as an alternative to *in vivo* tests should have their approaches validated through scientific studies to assess the reliability and relevance of the method for the given purpose [79].

Ex Vivo

An *ex vivo* model designates a methodology defined by experiments with living cells or tissues taken from an organism and placed in an external environment under controlled conditions [80-82]. The advantage of *ex vivo* experiments is that they are certainly useful to substitute the usage of whole animals as subjects, creating a high output research environment [80,81,83,84]. There are a gamut of studies in the literature employing *ex vivo* assays such as: *ex vivo* human gene therapy [85]; *ex vivo* cell cultivation such as within natural killers [86] and hematopoietic stem cells [87]; and analysis of organs *ex vivo* such as kidneys [88], spleen [89], heart, and lung [90].

Controversially, some authors still recognize an *ex vivo* method as a combination of the *in vivo* and *in vitro* models [78]. Indeed, due to the ambiguity with the terminology, researchers can easily be confused when classifying an *ex vivo* model. To clarify this issue, the well-known *ex vivo* gene therapy is a prime example. The *ex vivo* gene therapy is performed with cells isolated from a living organism, which are expanded/differentiated out of the living (with or without genetic modification) and further administered them back to the same or other living organism [91]. Although this technique clearly involves both *in vivo* and *in vitro* concepts, the best definition for this model should be *ex vivo*.

Other eminent *ex vivo* method is testing ocular corrosives on bovine corneas from slaughtered animals, a test recommended by the Organization for Economic Co-operation and Development (OECD) guideline [92]. An advantage of this assay is its speed, with results usually obtained within 24 hours [93]. The porcine cornea opacity permeability (PCOP) assay is other widely used approach

in the ophthalmic research field, which can be considered better in comparison to bovine corneas, since the porcine cornea better resembles the human cornea [94]. The mouse retina is also an excellent *ex vivo* model for determining autophagic cell function [95]. Also, the use of the same material can provide oxygen consumption information, showing the mitochondrial reserve capacity of photoreceptors [96].

Although *in vitro* and *ex vivo* methods can be considered in many cases a potential scientific tool to study different biological effects, sometimes the obtained results can indicate inconsistencies with *in vivo* models, indicating a potential misleading nature of *in vitro* and *ex vivo* procedures [97]. One example of *in vitro* and *ex vivo* experiment could be seen in Figure 2.

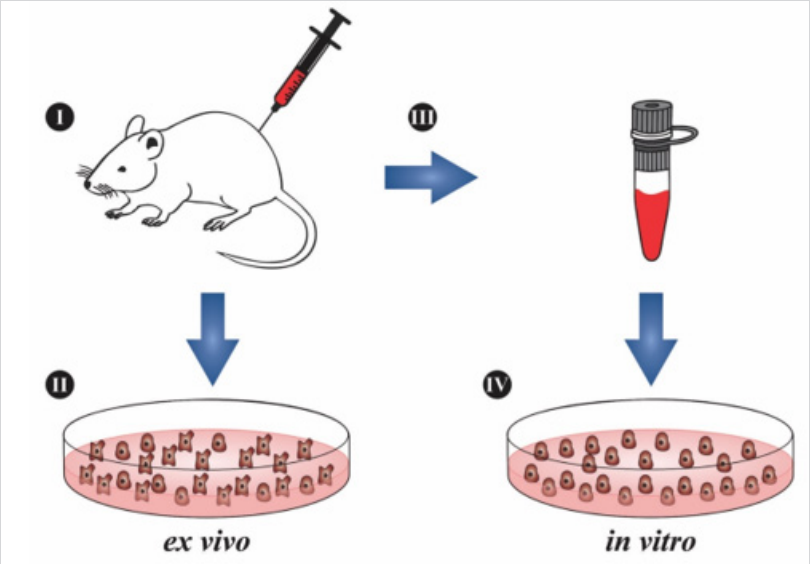

Figure 2: Difference between *in vitro* and *ex vivo* models. (I) Cells (e.g. leukocytes) are collected from an animal such as mouse; (II) differentiated cells are used right after animal collection, characterizing *ex vivo* assays; (III) cells taken from the animal undergo an immortalization process and are commercialized; (IV) immortalized commercial cells (cell lines) are used in assays characterizing *in vitro* models.

In Situ and *Ex Situ*

In situ models (i.e. on site or in position) can also been stated by researchers as in loco (i.e. in place), with both referring to studies performed inside its original place or "on site". *In situ* models are relevant in studying or identifying the active components and mechanisms in their place of origin, such as organs or tissues [98,99]. In medical research, this model is often used to evaluate genes and their respective translated proteins, such as a cytogenetic technique named fluorescent *in situ* hybridization (FISH), in which the genetic material can be assessed without being removed from the cell [100]. Basically, the FISH method uses fluorescently labelled probes that can bind to only those parts of a nucleic acid sequence with a high degree of sequence complementarity [101]. Therefore, FISH is considered a gold standard method for detection of gene alterations, a very useful tool for cancer diagnosis [102-104]. In situ neoplasia or carcinoma *in situ* is, by definition, the primal form of cancer and is characterized by being limited to the compartment corresponding to the cell of origin [105]. Notably, studies within *in situ* neoplasia have allowed the elucidation of tumor formation mechanisms [98,106,107]. In fact, in lymphomas, premalignant stages have been identified by evaluating *in situ* follicular lymphoma (FL) and mantle cell lymphoma (MCL) [107]. Several other studies regarding *in situ* carcinoma can be found elsewhere [106,108,109].

However, *in situ* models cannot be applied to all cell types, for example lymphocytes, which exhibit a circulating nature and cannot be studied in loco [98]. In the pharmacokinetics field, *in situ* models have also been explored for drug absorption tests [110-112]. In drug absorption evaluation through the intestinal barrier, the *in situ* intestinal model showed to be even more accurate than the *ex vivo* model [112,113]. This technique consists of a cannula introduction into the small intestine of anesthetized animals for the passage of the drug of interest to be evaluated. By maintaining innervation and blood flow preserved, these absorption models keep a faithful reproduction of what happens *in vivo* [110]. On the other hand, *ex situ* models are those performed outside the original place. For comparative purposes, in a study with bacterial cellulose used for tissue engineering cultures, the bacteria were cultured with *in situ* and *ex situ* modifications. Culture of bacterial cellulose *in situ* were characterized by adding in the medium other material such as an additive, while in the *ex situ* culture modification bacteria just received chemical treatment or absorption of other materials after the bacteria membranes have been formed in culture [114]. However, probably the most relevant *ex situ* method for biomedical sciences is the *ex situ* perfusion for organ preservation, which can restore circulation to regain the organ function (e.g. liver) prior to transplantation [115-117].

Ex situ techniques are also described in quality control of immunobiological products and in biodiversity conservation. In vaccine analysis, we find particle size distribution analysis, zeta potential and settling rate that are performed outside pre-filled syringes [118]. In biodiversity, the *ex situ* is used to describe gene banks, captive breeding, and botanical garden for biological diversity conservation. These methods are an essential part for the reintroduction of endangered species and have some restrictions such as high cost, employee demand and energy resources [119,120]. Examples of *in situ* and *ex situ* models could be seen in Figure 3.

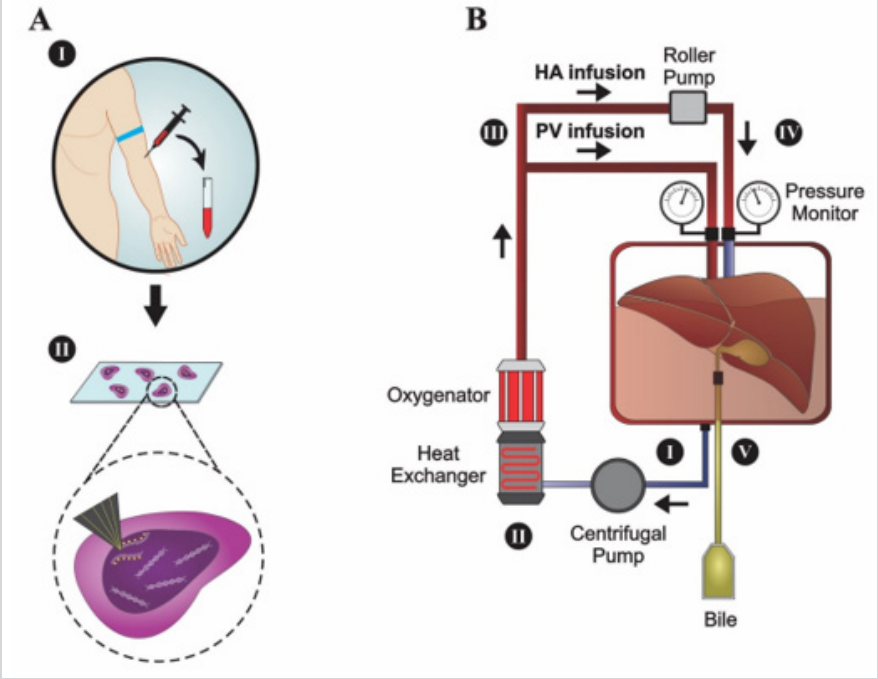

Figure 3: *In situ* and *ex situ* models. (a) Fluorescent *in situ* hybridization (FISH). The biological material is collected (I) and a fluorescent probe (II) is added to label the gene of interest. The material is analyzed using a fluorescence microscopic. (b) *Ex situ* organ perfusion. (I) The organ is placed in a container and submerged in a liquid suitable for infusions. This liquid flows to the centrifugal pump, heat exchanger and oxygenator (II). Through a bifurcation (III), the oxygenated liquid returns to the organ through the portal vein (PV) and part of it (IV) reaches a roller pump, to increase the pressure before returning to the organ through the hepatic artery (HA). (V) Container for the bile storage.

In Vivo

During human history and development, humans always took advantage from numerous scientific researches involving animals. Virtually, from the past century, every drug released on the market was dependent on the use of *in vivo* models in some step of their development [121].

The progress of specific and efficacious treatments of diabetes, several types of cancer, heart surgery, among others, were only possible using animals in scientific research [122]. A recent survey demonstrated that 44% of the population and more than 80% of medical students support the use of animals in research, although they also state that alternative methods should be applied, whenever possible, in order to reduce the number of animals for that purpose [123].

Overall, researchers seek to investigate organisms at multiple levels, starting from their molecules, to cells, tissues, organs, and up to their systems, both in disease and health conditions. For most of them, there is the option to conduct studies using *in silico* or *in vitro* models, such as molecular modeling and cell line culture, respectively. Cell line culture, specifically, has largely evolved in the past 20 years, and became a usual approach to mimic the complex structures of tissues, currently playing key roles in research. In this regard, several animal studies can be replaced due to the advent of such technology [124,125]. Nonetheless, the understanding of physiological processes and systemic interactions still requires *in vivo* experiments.

In vivo model (i.e. within the living) describes a methodology where the whole subject is studied while alive. Although *in vivo* models can be performed with non-animals, such as plants and seeds [126], *in vivo* experiments using animal models are the most used, reaching more than 100 million animal experiments per year in the world (e.g. Great Britain performed 1,078,738 experimental procedures just in mice in 2018) [127,128]. *In vivo* animal assays are carried out when specific situations are not suitable to be done in humans, due to a possible risk to their physical and/or physiological integrity. The animal use in research is not only due to their similarity with human physiology, but also because human diseases may also affect other animal species [129]. Additionally, it is possible to have *in vivo* models with specific mutations that directly cause or strongly predispose the animal to a desired anomaly or disease, being referred to as "experimental models of a disease" [129-132].

Research with animals have the purpose of providing experimental data in one living organism to study a phenomenon in another species, aiming to obtain preclinical data to determine how a hypothesis would work on humans [133]. On the other hand, the results obtained using animals are not necessarily reproducible in humans. Despite great similarities that a species may have with humans, genetic differences such as gene families, redundancies, and regulation of gene expression patterns may influence the variable responses [122].

Although massive worldwide investments in drug development, the overall success rate of new drugs, especially on clinical trials, remains low. One possible explanation relies on the preclinical research phase, when 'inappropriate animal models' are chosen because of lacking a predictive model, which leads to non-extrapolating conclusions [134]. In this context, it is fundamental to select the correct animal model according to the objectives of the proposed research [134].

Although rodents (rats, mice, and guinea pigs) are mostly used due to their shorter lifespans, which creates the possibility of producing many generations [135], there are many other available animal models, such as insects (*Drosophila* ssp.) [136], fish (Danio rerio, or zebrafish) [137], nematodes (*Caenorhabditis elegans*) [138], frogs (*Xenopus* ssp.) [139], rabbits [140], horses [141], dogs [142], cats [143], pigs [144], and even non-human primate models [145], among others (Figure 4). Some examples of preclinical animal models and their use in research are listed in Table 1.

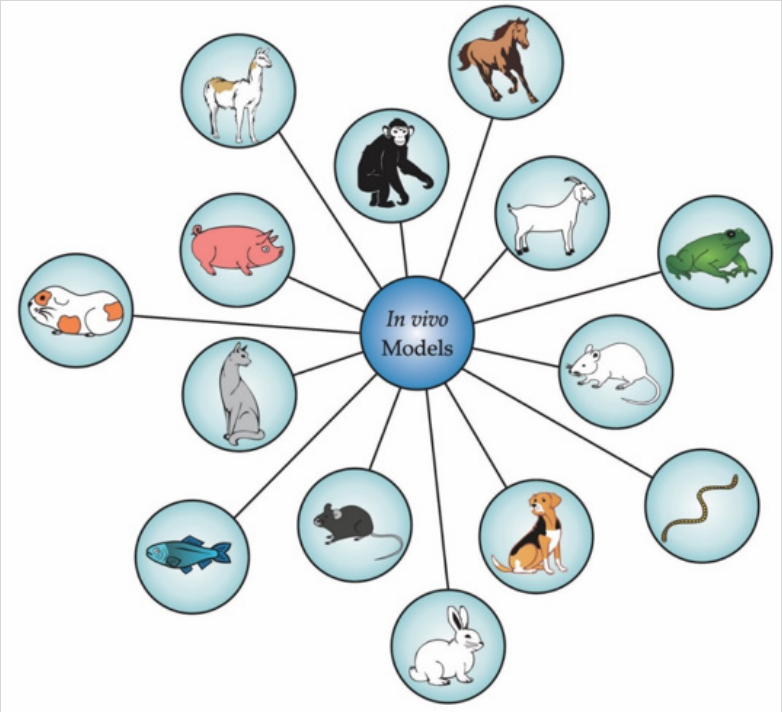

Figure 4: Main *in vivo* models used in preclinical research.

Table 1: *In vivo* models in preclinical research.

In vivo model	Main Species Employed	Models in Research	Reference
Cat	*Felis catus*	Ophthalmology, Type-2 Diabetes	[170], [171]
Dog	*Canis familiaris*	Osteoarthritis, Periodontology	[172], [173]
Fish	*Danio rerio*	Drug development screening, Behavioral studies, Cancer therapy, Autoimmune diseases, Nanomedicine	[174], [175], [176], [177], [178]
Flies	*Drosophila melanogaster*	Drug development screening, Cancer therapy	[179], [180]
Frog	*Rana pipiens*	Analgesia and nociception, Electrophysiology, Multiple sclerosis, Teratogenesis	[181], [182]
	Xenopus laevis		[183], [184]
Goat/sheep	*Ovis aries*	Osteoporosis, Bone tissue engineering	[185], [186]
	Capra aegagrus hircus		
Guinea pig	*Cavia porcellus*	Cardiovascular disease, Pharmacological characterization	[187], [188]
Horse	*Equus ferus caballus*	Depression, Heterologous antiserum and antivenom generation	[189], [59,190]

Llama	*Lama glama*	Nanobody (VHH) generation	[191]
Mice	Wild derived strain:	Cancer therapy, Drug development, Hybridoma technology, Toxicological evaluation, DNA vaccines, Nanotechnology, Inflammation, Chronic stress, Behavioral studies and depression	[192], [193], [194]
	Mus musculus		[62,195], [196]
	Inbred strains:		[197], [198]
	BALB/c, C57BL/6		[199], [200]
Nematode	*Caenorhabditis elegans*	Drug development screening, Toxicological evaluation	[201], [202]
Non-human primates	*Callithrix jacchus*	Neuroscience research, HIV, Anxiety, Tuberculosis	[203], [204]
	Macaca mulatta		[205], [206]
	Macaca fascicularis		
Pig	*Sus scrofa domesticus*	Wound healing, skin graft, Pharmacokinetics	[207], [208]
Rabbit	*Oryctolagus cuniculus*	Atherosclerosis, Immunogenicity, Bone implants	[140], [209], [210]
Rat	*Rattus norvegicus domestica*	Anxiety and depression, Toxicological evaluation, Cancer therapy, Hypertension, Neurodegenerative diseases, Drug discovery	[211], [212]
	Wistar		[213], [214]
	Lewis		[215], [216]

Non-human primates have been extensively used as models in animal research, due to their close phylogenetic relationship to humans, with validated similarities in terms of behavioral and biochemical activities, as well as gene expression patterns [146]. Based on that, some non-human primates are considered key *in vivo* models for specific research fields, including studies with the acquired immunodeficiency disease syndrome (AIDS), autoimmune diseases, Parkinson's disease, hepatitis, diabetes, physiological and psychiatric disorders, transplants, toxicological effects, dentistry, drug abuse and vaccine development [133,147].

Nevertheless, there are many regulatory requirements and policies to perform experiments on non-human primates, especially because they are large animals, intelligent, social, long-lived, and non-domesticated animals [148]. Therefore, non-human primates should only be used in specific cases, when the less-sentient species do not meet the requirements of the research.

PICKING THE BEST RESEARCH MODEL

Selecting the best research model can be a real challenge. Based on the efforts to 3Rs principle (Reduce, Refine and Replace the use of animals), many *in vitro* and *in silico* models have been explored as alternatives to *in vivo* methods, such as organs-in-a-chip and toxicity prediction, respectively. Nevertheless, these alternative models should be mechanistically based on the *in vivo* process, scientifically supported, and based on well-known responses described *in vivo*, which means that the model should be validated before being adopted. Fortunately, the availability of *in vivo* alternative tests has increased intensely, and many are very attractive in cost and time [149].

Researchers can obtain information about the alternative validated methods through different regulatory agencies [150-152]. Based on the above, method validation is vital to check the safety and efficacy of the research. In concern to the biopharmaceutical drugs, many regulatory organizations have addressed this issue in the chemical and pharmaceutical industry. For instance, the analytic validation can be assessed at two levels.

1. The pre-study validation that aims to show the method can achieve its objectives; and

2. In-study validation that verifies if the method remains valid over time by including quality control samples in routine runs [153,154].

However, the main challenge in biomedical sciences is when the model cannot be compared to humans. Animal models have supported critical advances in biomedical research, offering deep insights into several diseases. But they have been less successful as a basis for advancing human health as the failure of translation from animal models to human patients has often been disappointing [155].

A common criticism of model-based biomedical research is that while we have gotten very good at curing mouse models, we have made much less progress for human patients and, although the assumption that the animal models are a good proxy for humans is a central tenet of biomedical research, it is not always a reliable one [156-158]. For instance, it is well-known the gap between human and murine inflammation responses. Thus, how can we compare mice models of human immune-related diseases (e.g. diabetes, asthma, and multiple sclerosis)? There are many animal data failing to predict human responses to potential immunological therapies [159].

Choosing of the best experiment to validate a hypothesis is not the only issue for researchers. There is no consensus in the scientific community for the model classification. Misunderstandings with *in vitro*, *ex vivo*, *in situ*, *ex situ*, and *in vivo* models are very frequent, simply because there is no article or review giving satisfactory information about differentiating these models. Although most academy agrees that in vivo models are those conducted in animals, the literature presents articles in which assays conducted out of animals (e.g. *ex vivo* and *in vitro*) are referred as *in vivo*. For instance, the chick chorioallantoic membrane (CAM) assay can be defined by researchers either as an *ex vivo* [160] or as an *in vivo* [161] approach.

However, since the assay is developed in the alive chick embryo (which last 21 days to develop), should fit better to classify the assay as an *in vivo* model [162].

Furthermore, it is easy to come across hundreds of research articles, even in highly reputed journals, where cell line culture experiments are referred to as both *in vitro*, and sometimes, *in vivo* [163-166]. Thus, which research model should be the best to classify cultures performed with cell lines? Following the previous definition, we encourage researchers to refer as *in vitro* all experiments developed with cell lines. Histological studies also easily generate confusion among researchers since they can be classified as *in vivo* [167], *ex vivo* [168], as well as *in vitro* [169] models. Basically, the differences between the models are based on the way that the tissue was obtained. *Ex vivo* and *in vitro* histology should follow Sections. On the other hand, *in vivo* histology is a peculiar model, where the tissue is accessed through an intravascular ultrasound radiofrequency technique, named also virtual histology.

CONCLUSION

As can be seen above, researchers can get very confused to pick and classify their research models since there are no guidelines for choosing and classifying them so far. In addition, these models and their classification may vary considerably among the several scientific fields, different research organizations, and individual investigators. This review is the first to clarify the main definitions of the principal research models used in biomedical sciences with marked examples, aiming to guide and align researchers during their experimental practices.

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AUTHORS CONTRIBUTION

ISO, GMAS, FAC, ELPJ, IGF and UZ wrote the review, FAC provided figures and wrote the review and MBP was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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