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Advancements in Alternative Methods to Animal Experimentation in Preclinical Pharmacology

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ABSTRACT

The development of reliable and ethical research methodologies has become essential in modern drug discovery, toxicology, and biomedical sciences. Traditional animal experimentation, while valuable, faces increasing ethical concerns, high costs, and limitations in predicting human responses. Consequently, alternative approaches such as Quantitative Structure–Activity Relationship (QSAR) models, in silico methods, Physiologically Based Pharmacokinetic (PBPK) modeling, 2D cell culture systems, organoids, microorganisms, zebrafish (Danio rerio), invertebrates, and vertebrate models have gained significant attention. QSAR and in silico techniques facilitate the prediction of biological activity and toxicity of compounds, reducing the need for extensive laboratory testing. PBPK models provide mechanistic insights into drug absorption, distribution, metabolism, and excretion. Cell culture systems and organoids offer physiologically relevant platforms for studying disease mechanisms and drug responses. Microorganisms, zebrafish, invertebrates, and lower vertebrates serve as cost-effective and ethically favorable experimental models. Furthermore, regulatory frameworks such as the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) promote the principles of Replacement, Reduction, and Refinement (3Rs) to ensure ethical research practices. This review summarizes the principles, applications, advantages, and limitations of these methodologies and highlights their role in advancing predictive, efficient, and humane biomedical research.

Keywords: Quantitative Structure–Activity Relationship (QSAR) models; In-silico methods; 2D cell culture systems; Organoids; PBPK models; CPCSEA; invertebrates, and vertebrate models

INTRODUCTION

The Prevention of Cruelty to Animals (PCA) Act, 1960 empowered the Central Government to establish the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) to ensure that animals are not subjected to unnecessary pain or suffering during experiments. To regulate animal experimentation, the Government introduced the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, which were later amended in 2001 and 2006.(1)

3Rs Principles and Their Implementation (CPCSEA)

1. Replacement:

Use alternatives to animals whenever possible, such as in vitro methods, computer models, or other non-animal techniques. Researchers must justify animal use before approval.

2. Reduction:

Minimize the number of animals used by employing proper study design and statistical planning, ensuring valid results with the fewest animals necessary.

3. Refinement:

Improve animal welfare by reducing pain, distress, and suffering through better housing, humane handling, and the use of anesthesia and analgesia during procedures.

CPCSEA Implementation:

The CPCSEA incorporates the 3Rs into IAEC SOPs, ensuring ethical animal research while promoting animal welfare.

Traditional two-dimensional (2D) cell culture systems and animal models have been widely used in biomedical research. However, they cannot accurately mimic the complex environment of human tissues and organs. Organ-on-a-Chip (OOAC) technology was developed to overcome these limitations by creating miniature models that closely resemble human physiological conditions. It is considered a promising alternative to animal testing and can improve drug discovery and disease research (2). Design Concept of OOAC combines micro engineering, microfluidics, and cell biology to recreate the natural environment of human organs. The system controls both internal and external factors affecting cells, allowing them to behave similarly to cells inside the human body.(3)

2. BACKGROUND

✓ **OOAC (organ on a chip) TECHNOLOGIES:**

History of Organ-on-a-chip

The concept of "organ-on-a-chip" was pioneered in 2010 by Huh et al., who published the first study on a "lung-on-a-chip." They defined these systems as microfluidic in vitro environments designed to replicate the physiological functions of an organ's basic units. Following this, a 2012 public-private partnership tasked Donald Ingber 's lab with developing ten different human organ chips. According to Hamilton, models for the liver, intestine, and lung have already been established, with current research focused on next-generation chips for the brain, kidney, and skin[5].

Organ-on-a-Chip (OOC) Design Concept:

Cell culture systems require precise control of both the external and internal cellular environment to maintain normal cell function. Organ-on-a-Chip (OOC) technology integrates micro engineering techniques with cell biology to recreate physiological conditions similar to those found in the human body.

The design of an OOC device allows accurate control of important factors such as fluid flow, mechanical forces, and chemical concentration gradients. These systems can also generate dynamic conditions, including fluid shear stress and mechanical stretching, which are essential for mimicking natural organ functions. Additionally, proper cell patterning and organization within the chip help reproduce complex physiological processes more realistically.

By closely simulating the in vivo environment, OOC platforms provide a powerful tool for studying organ function, disease mechanisms, and drug responses.[4]

The main components of OOAC are:

1. Fluid Shear Force

Microfluidic systems continuously supply nutrients and remove waste products.

The flowing fluid produces shear stress, similar to blood flow in the body.

This mechanical force helps maintain normal cellular functions and supports cell signaling and organ development.

2. Concentration Gradient

Biological molecules are distributed in varying concentrations within tissues.

OOAC creates stable chemical gradients to study processes such as: Angiogenesis (formation of blood vessels), Cell migration, Tissue invasion.

These gradients help mimic the real physiological environment.

3. Dynamic Mechanical Stress

Human organs experience different types of mechanical forces, such as:

Blood pressure

Lung expansion and contraction

Bone pressure

OOAC uses flexible membranes and microdevices to reproduce these forces, which are essential for cell growth and differentiation.

4. Cell Patterning

Cells in the body are arranged in an organized manner to perform specific functions.

OOAC uses techniques such as: Surface modification, Templates and 3D printing.

These methods help arrange cells in a way that closely resembles natural tissues[3].

Advantages of Organ-on-Chip Technology

- Low manufacturing cost.
- Allows testing of drugs at different concentrations.
- Mimics the real human cell environment (oxygen, temperature, pH) more accurately than Petri dishes.
- Uses microfluidics, making it an innovative research tool.
- Easy to use, portable, small in size, and suitable for mass production.

Disadvantages of Organ-on-Chip Technology

- Analysis may be affected due to adsorption of substances on the inner surfaces of the chip.
- Low Reynolds number causes laminar flow, leading to poor mixing of fluids.

➢ **Liver OOAC**

The liver is a vital organ responsible for maintaining many important physiological functions in the body. It has a remarkable ability to regenerate and recover from physical or chemical injury. However, certain drugs and diseases can cause severe or permanent liver damage. Research on liver toxicity has been challenging due to the lack of accurate laboratory models that mimic human liver function.

Traditionally, drugs were tested on animals before being used in humans, but some medications still produced serious side effects that were not detected during animal testing. To overcome this limitation, the Organ-on-a-Chip (OOC) technology has been developed. In a liver-on-chip model, liver cells are cultured within a microfluidic chip that replicates the liver's natural environment. Researchers can then study both the therapeutic and toxic effects of drugs on these cells.

Liver-on-chip systems provide a more reliable platform for evaluating drug safety and investigating hepatotoxicity, helping to improve drug development and reduce dependence on animal testing.[6]

- **LUNG OOAC**

Lung-on-a-chip is an advanced microfluidic device that mimics the structure and function of the human lung. It provides a realistic platform for studying lung diseases, drug responses, and toxicity, reducing the need for animal testing.

Pulmonary diseases are among the leading causes of death worldwide, creating a need for improved treatment strategies. Researchers at the Wyss Institute, Harvard University, led by Huh et al., pioneered the development of the lung-on-a-chip model. In 2015, they designed a dynamic microenvironment that closely replicated the human alveolar– capillary interface. This model was successfully used to study nanoparticle-induced toxicity by measuring the production of reactive oxygen species (ROS) and to simulate pulmonary edema in human lungs.

Researchers have also used lung-on-a-chip models to investigate the harmful effects of cigarette smoke. Benam et al. developed a breathing lung-on-a-chip that simulated inhalation and exhalation of smoke. Their findings closely matched animal studies and revealed smoking-induced changes in gene expression along with the discovery of new biomarkers.

Shrestha et al. developed an open-well lung-on-a-chip model that recreated the in vivo airway environment. The model was used to evaluate cell viability, mucus secretion, membrane permeability, and P-glycoprotein (P-gp) expression. It showed promising applications in toxicology testing, permeability studies, and pulmonary drug delivery. The effects of cigarette smoke on inflammatory cytokines such as IL-6 and IL-8, as well as the anti-inflammatory effects of budesonide, were also successfully studied using this model.

During the COVID-19 pandemic, the human airway-on-a-chip was used to evaluate the repurposing of existing antiviral drugs. The model simulated viral infection, cytokine production, and immune cell interactions using human bronchial epithelial and pulmonary endothelial cells. It proved useful for rapidly assessing potential COVID-19 treatments and understanding disease mechanisms.

Key Applications:

Drug screening and development

Pulmonary disease modeling

Nano toxicology studies

Smoking-related lung disease research

Pulmonary drug delivery testing

COVID-19 drug repurposing

Reducing dependence on animal experiments[7].

- **KIDNEY OOAC**

The kidneys filter waste products and excess water from the blood, producing urine while maintaining the body's balance of sodium, potassium, and calcium. They also secrete hormones that regulate blood pressure and stimulate the production of red blood cells.

Jang and colleagues developed the first multilayered microfluidic system that simulated kidney filtration using mouse kidney cells. This device was later adapted to culture human kidney cells and became one of the earliest platforms for studying the toxicity of primary kidney epithelial cells.

Musah and co-workers later designed a kidney-on-a-chip that closely replicated the structure and function of the kidney's capillary wall. The device incorporates vacuum chambers on both sides, allowing mechanical pressure to be applied to the cell layer. Since then, kidney-on-a-chip technology has been widely used for disease modeling, drug screening, and toxicology studies.

Kim and colleagues evaluated the nephrotoxicity of gentamicin under two different pharmacokinetic conditions using a kidney-on-a-chip model. Their findings showed that continuous drug infusion caused less kidney toxicity than bolus injection. They also highlighted the potential of microfluidic cell culture systems for future pharmacokinetic and toxicity research [8].

HEART OOAC

Heart-on-a-Chip (HOC) is an advanced and cost-effective technology used to develop engineered cardiac tissues for drug discovery and clinical research. It creates a biomimetic microenvironment that supports the growth, maturation, and real-time monitoring of heart tissue. The successful development of HOC depends on the integration of three key components— scaffolds, stimulation systems, and sensors (the "3S" components)—which improve tissue maturation and enable accurate assessment of cardiac function. This review summarizes the structures and fabrication methods of these components, provides recommendations for their design and manufacturing, and discusses the major challenges and future prospects of HOC platforms with integrated 3S technologies [9].

INTESTINE OOAC

The 3D layout is what makes an intestine-on-a-chip actually mimic the gut vs just being cells in a channel.

Here’s how the 3 main structures work:

I. 2D Sandwich structure:

The classic design you mentioned. Two PDMS micro channels stacked with an ECM-coated porous membrane in between.

- Top channel: Intestinal epithelial cells form a monolayer + microvilli
- Bottom channel: Endothelial cells + flow to mimic blood vessels
- Why it’s used: Easy to build, gives you tight junctions + barrier function.

You can apply cyclic stretch to mimic peristalsis.

- Limitation: Still basically 2D. Villi/crypts are flat, so absorption area and cell differentiation aren’t fully physiological.

Planar parallel structure:

Channels sit side-by-side instead of stacked, separated by a vertical porous membrane or pillars.

- Layout: Epithelium on one side, endothelium/immune cells on the other, sharing the same plane
- Why it’s used: Easier optical access + sampling. Great for co-culture studies, microbiome interaction, and drug transport assays because both channels are on one focal plane.
- Limitation: Membrane is vertical, so gravity-driven cell seeding is trickier. Still lacks 3D villi architecture.

3D Scaffold structure:

Tries to recreate actual gut geometry with villi and crypts.

- How: Hydrogel scaffolds, micro pillar arrays, or laser-ablated ECM form 3D villus-like structures. Cells are seeded and they self-organize along the topography.

- Why it's used: Most biomimetic. You get real apical/basolateral polarity, mucus layer gradients, stem cell niches in "crypts", and more accurate absorption/metabolism data.

- Limitation: Fabrication is complex. Imaging + fluid dynamics are harder to control vs 2D.[10]

BRAIN OOAC

Brain-on-a-chip is a microfluidic model that reproduces the blood-brain barrier (BBB), which protects the brain and controls the entry of substances from the blood. This model allows researchers to study drug transport across the BBB and brain diseases more effectively. The flowing fluid inside the chip mimics blood circulation, helping brain cells grow and function normally.

Advantages:

Provides a realistic model of the blood-brain barrier.

Better predicts drug permeability than static cell cultures[3].

- **MICRO FLUDIC LIVER CHIP**

Microfluidic liver chip models (Liver-on-a-chip) bridge the gap between simple 2D monolayers and complex in vivo environments. They use micro engineering techniques to precisely control mechanical, chemical, and fluidic dynamics. These systems simulate hepatic tissue by providing continuous nutrient delivery, fluid shear stress, and accurate spatiotemporal cell-to-cell interactions [11].

Advantages of Microfluidic Technologies in Organ-on-a-Chip Models:

- Recreate the natural, directional flow of fluids found in human organs.

- Ensure a continuous supply of nutrients and efficient removal of waste products.

- Generate controlled concentration gradients, making drug dosing and response studies more accurate.

- Support the arrangement of multiple cell types in layers to closely mimic the structure and function of real organs.

- Utilize human-derived cells, providing results that are more physiologically relevant than traditional animal models.

- Enable precise genetic modifications (such as gene deletion or mutation) to study specific disease mechanisms in isolation without interference from other genetic changes[12].

- **ORGAN ON A CHIP MICRO ENGINEERING**

Organ-on-a-Chip Micro engineering Organ-on-a-chip (OOC) engineering has garnered significant interest from pharmaceutical companies, regulatory bodies, and national defense agencies. This is evidenced by the emergence of at least 28 OOC companies within just seven years (Balijepalli and Sivaramakrishnan, 2017; Han et al., 2014).The technology primarily focuses on three major areas to replicate organ function. Multicellular Interfaces: Recreating complex boundaries, such as those found in blood vessel networks, the lungs, and the gut. Tissue-Level Organization: Structuring parenchymal cells to mimic solid organs like the liver and heart. Systolic and Diastolic Systems: Developing systems that act as tissue barriers, similar to the functions of kidneys and the liver.

✓ IN SILICO METHODS

➢ Quantitative Structure–Activity Relationship (QSAR):

QSAR is a computational method that relates the chemical structure and physicochemical properties of compounds to their biological activity. Developed by Hansch and Fujita (1962), it is widely used in drug discovery to predict activity, identify new drug candidates, and optimize lead molecules.

Uses molecular descriptors representing structural and chemical properties.

Models are trained using compounds with known biological activity.

Helps reduce experimental time and cost in drug development.

3D-QSAR was developed to overcome limitations of traditional QSAR by incorporating three-dimensional molecular information.

Common QSAR tools include QSAR-Co and Open3DQSAR.(13)

➢ PBPK Modeling in Drug Research and Development :

- Physiologically Based Pharmacokinetic (PBPK) models consist of compartments representing body organs connected through the blood circulation system. Each compartment is characterized by species-specific tissue volume and blood flow rate.
- Tissues are modeled using either.
- Perfusion-rate-limited kinetics: common for small, lipophilic drugs where blood flow limits tissue uptake.
- Permeability-rate-limited kinetics: common for larger or hydrophilic drugs where membrane permeability limits uptake.
- After distribution and possible metabolism within organs, drugs leave tissues through the venous blood flow and continue circulating or are eliminated.

Applications:

- PBPK (Physiologically Based Pharmacokinetic) modeling is used throughout drug development, from early discovery with limited data to late-stage development with extensive clinical information. Its major applications include:
- Supporting Regulatory Decisions – Provides scientific evidence for regulatory submissions and communications.
- Guiding Clinical Development – Helps optimize dosing strategies, predict drug interactions, and support clinical trial design.
- Understanding Drug Behavior Mechanistically – Explains clinical observations by linking drug pharmacokinetics to physiological processes.
- These applications improve decision-making and enhance the efficiency of drug development.(14)

➢ Docking based virtual screening:

Docking-based virtual screening is a computational technique used in drug discovery to identify potential drug candidates by predicting how a ligand binds to a target receptor. It evaluates binding modes, molecular interactions, and binding affinity between the ligand and receptor. Several molecular docking programs are commonly used, including Auto Dock, GOLD, Glide, Swiss Dock, Dock Thor, CB-Dock, and Molecular Operating Environment (MOE). Before docking, protein structures obtained from X-ray crystallography or NMR spectroscopy should be carefully prepared because they may contain missing hydrogen atoms, incomplete side chains, incorrect protonation states, or flipped residues. Proper preparation of the 3D structure improves the accuracy of docking results.

Molecular docking has three major objectives:

Predict how a ligand binds to the active site of a receptor.

Screen large compound libraries to identify promising drug candidates.

Estimate the binding affinity of ligands using scoring functions.

An effective docking algorithm should explore all possible ligand binding conformations. The concept of molecular docking originated from Emil Fischer's "lock-and-key" model, which assumed that proteins and ligands were rigid structures. Modern docking methods recognize that both proteins and ligands are flexible molecules. Therefore, current docking software incorporates varying degrees of molecular flexibility to produce more realistic and reliable predictions of ligand–receptor interactions[16].

2D and 3D cell cultures:

2D Cell Culture

- Cells grow as a monolayer attached to a flat plastic surface.
- Advantages: Simple, inexpensive, reproducible, easy to maintain, and compatible with many functional assays.

Limitations:

- Poorly mimics natural tissue and tumor architecture.
- Reduced cell–cell and cell–extracellular matrix (ECM) interactions.
- Altered cell morphology, polarity, gene expression, signaling, and metabolism.
- Uniform exposure to oxygen and nutrients unlike in vivo conditions.
- Usually supports only a single cell type, lacking the tumor microenvironment.

3D Cell Culture

Cells grow in three-dimensional structures that better resemble living tissues and tumors.

Types:

- Suspension cultures on non-adherent plates (spheroids)
- Gel-based cultures (soft agar, Matrigel)
- Scaffold-based cultures (collagen, alginate, silk, etc.)

Advantages:

- Mimics in vivo tissue architecture and microenvironment.
- Maintains cell morphology, polarity, differentiation, and phenotype.
- Preserves realistic cell–cell and cell–ECM(Extra cellular matrix) interactions.
- Produces more physiologically relevant gene expression, signaling, metabolism, and drug responses.
- Useful for studying cancer stem cells, invasion, metastasis, angiogenesis, drug resistance, and radiation response.

Limitations:

- More expensive and time-consuming.
- Lower reproducibility and more complex analysis.
- Difficult cell recovery and imaging in some systems.
- Experimental conditions require careful optimization.
- Co-Culture Systems
- Grow multiple cell types together to mimic tissue complexity.
- Can be direct (cells in contact) or indirect (separated by a barrier).
- Allow study of cell–cell communication, microenvironment interactions, and paracrine signaling.

More physiologically relevant but technically challenging due to multiple variables(17)

Deciding Between 2D and 3D Cell-Based Assays:

- Start by defining the objective of the experiment. If a simpler, low-cost 2D assay can answer the research question, it may be the most practical choice.
- If greater physiological relevance is needed, consider 3D cultures and select the model that best fits the study requirements.
- 2D approach (cells grown on flat surfaces with some 3D characteristics) can provide advantages of both 2D and 3D systems.
- Consider resources, budget, and throughput: 2D assays are simpler, cheaper, and higher-throughput, while 3D assays are more biologically relevant but costlier and more complex.
- Combining 2D and 3D approaches can often provide the most comprehensive and representative results.(18)

✓ **ORGANOIDS**

Organoids are 3D cell cultures derived from primary tissues or stem cells that can self-renew, self-organize, and mimic the structure and function of real organs.

Key Features:

- Resemble the composition and architecture of native tissues.
- Provide biologically relevant models that closely mimic in vivo conditions.
- Can be cultured long-term, expanded indefinitely, and cryopreserved for future use.

How Are Organoids Generated?

- Organoids are developed from primary tissues or pluripotent stem cells such as iPSCs and ESCs(embryonic stem cells) by providing suitable physical and biochemical signals.
- Physical cues: Support cell attachment, growth, and survival (e.g., collagen, fibronectin, laminin).
- Biochemical cues: Regulate cell proliferation, differentiation, and self-renewal through signaling pathways (e.g., EGF, FGF10, WNT3A(wingless –type MMTV integration site family member3A), R-spondin, Retinoic acid, Noggin, Activin A).

- These cues enable cells to self-organize into organ-like 3D structures called organoids.(19)

➢ **Human brain organoids:**

Human brain development is a complex and lengthy process influenced by both genetic and environmental factors. While genetics plays a key role in brain formation and neurodevelopment, the molecular mechanisms underlying many neuropsychiatric disorders remain poorly understood. Advances in human stem cell culture and differentiation have enabled the creation of in vitro models that closely mimic human brain development. These models provide valuable tools for studying diseases, understanding biological processes, testing therapies, and reducing reliance on animal models, thereby advancing regenerative medicine, drug discovery, and neuroscience research.(20)

➢ **Renal organoids:**

Retinal organoids derived from human induced pluripotent stem cells offer significant potential for retinal disease modeling and drug screening. However, challenges such as incomplete cell composition and immature functionality remain. Future research focuses on improving culture methods and integrating organ-on-a-chip technologies to enhance organoid maturation and better mimic native retinal tissue.(21)

➢ **Kidney organoids:**

Kidney organoids are innovative models for studying kidney biology and disease. This review highlights their applications in kidney development, disease modeling, drug testing, and precision medicine. It also discusses recent advances such as single-cell RNA sequencing and microfluidic devices, along with their achievements, limitations, and potential for future research and clinical therapies.(22)

➢ **Liver organoids:**

Liver organoids are three-dimensional (3D) structures derived from pluripotent stem cells, progenitor cells, differentiated cells, or tumor tissues that self-organize to mimic the architecture and functions of the liver. A standardized classification developed by experts improves communication and reproducibility in organoid research. Liver organoids are broadly categorized into tumor organoids (tumoroids), epithelial organoids (hepatocyte, cholangiocyte, and non-parenchymal organoids), and multi-lineage organoids that contain both parenchymal and supporting cells, closely resembling the liver's natural organization.(23)

➢ **Intestinal organoids:**

Intestinal organoids derived from both adult stem cells (ASCs) and pluripotent stem cells (PSCs) are based on the ability of intestinal stem cells (ISCs) to self-renew and self-organize. When cultured in an extracellular matrix under suitable conditions, ISCs form polarized spheroids with an inner lumen, where the apical surface faces inward and the basal surface faces the matrix. Differences in stem cell source, culture conditions, and cellular composition give ASC- and PSC-derived organoids distinct advantages and limitations for clinical applications.(24)

✓ **Invertebrates and lower vertebrates**

Invertebrate animal models are non-vertebrate organisms, such as the fruit fly Drosophila and the nematode Caenorhabditis elegans, used in biomedical research to study biological processes and molecular pathways while reducing ethical concerns related to vertebrate animal use. Invertebrate models emerged as ethical alternatives to vertebrate animals and include insects (e.g., Drosophila), nematodes (e.g., Caenorhabditis elegans), and aquatic organisms such as planarians, crustaceans, and molluscs.(25)

✓ **Caenorhabditis elegans (C. elegans)**

- A simple invertebrate nematode widely used in biomedical research.
- Helps identify genes and molecular pathways involved in human diseases.
- Has a simple nervous system with a defined number of neurons, making it useful for developmental and neurological studies.
- Easy to culture, inexpensive, and genetically tractable.

- Limitation: lacks the complex organs and central nervous system found in vertebrates.

✓ **Zebrafish (Danio rerio)**

- A vertebrate model organism that bridges invertebrate and mammalian disease models.
- Shares many genetic and physiological similarities with humans.
- Transparent embryos allow direct observation of organ and nervous system development.
- Produces large numbers of embryos, enabling high-throughput studies.
- Widely used to study development, genetics, and human diseases due to its vertebrate organ systems and genetic tractability.(26)

✓ **Micro organisms**

- Microorganisms are microscopic, generally unicellular organisms belonging to diverse groups. According to the five-kingdom system, they include members of Monera, Protista, and Fungi, while the modern three-domain system classifies them into Bacteria, Archaea, and Eukarya. Due to their simplicity, rapid growth, and vast diversity, microorganisms serve as important model organisms for genetic studies, genetic engineering, and industrial applications.

- Accurate research on microorganisms depends on their purity and correct identification. Therefore, isolation is essential. Microbial isolation involves separating a single species from its natural environment and growing it in a pure culture on a sterile medium. Pure cultures allow researchers to study specific characteristics, identify organisms, and obtain experimental material. Properly maintained cultures can survive for years and serve as valuable reference collections for taxonomic and research purposes.(27)

✓ **ADVANCES IN AI MODELS**

AI-powered toxicity prediction models revolutionize drug safety assessments by replacing time-intensive, cross-species animal experiments with scalable, accurate, and human-centric computational screening. They reduce late-stage clinical trial failures and development costs while improving personalized medicine and addressing ethical concerns regarding animal testing .Key advantages of implementing artificial intelligence and machine learning in toxicity prediction include :Human-Specific Dataset Utilization: Unlike animal models, AI relies on human-derived data like electronic health records (EHRs) and omics (genomic and proteomic) datasets, leading to higher translational accuracy.

Personalized Toxicity Predictions: AI accounts for individual variability by analyzing patient demographics, genetic profiles (e.g., CYP450 enzyme variations), and environmental factors .Early Safety Assessment: AI algorithms allow researchers to filter out potentially toxic compounds virtually before conducting in vitro or in vivo experiments, saving significant time and resources .Scalability: Machine learning models can quickly process massive datasets containing drug structures, biological targets, and toxicity end points .Ethical Improvements: By improving predictive capabilities through big data, AI reduces the reliance on and use of conventional cellular and animal models[15].

Conclusion

- The integration of alternative and advanced research methodologies has transformed biomedical and pharmaceutical research by improving efficiency, predictive accuracy, and ethical compliance. Approaches such as QSAR, PBPK modeling, in silico techniques, 2D cell cultures, and organoids provide valuable tools for understanding drug behavior and biological responses while reducing dependence on animal testing. Experimental models including microorganisms, zebrafish, invertebrates, and vertebrates continue to contribute significantly to toxicity assessment, disease modeling, and therapeutic development.

- CPCSEA guidelines further reinforce the adoption of ethical practices through the implementation of the 3Rs principle. Although no single model can completely replace traditional animal studies, the combined use of computational, cellular, and alternative organism-based approaches offers a comprehensive strategy for modern research. Future advancements in these technologies are expected to enhance translational relevance, reduce animal usage, and accelerate the development of safer and more effective therapeutic interventions.

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