Int. J. Sci. R. Tech., 2026 3(5)
A Multidisciplinary peer-reviewed Journal
www.ijsrtjournal.com [ISSN: 2394-7063]

Original Article

Beyond The Cage: Advancing Science In In-Vivo, In-Vitro & In-Silico Models

Gayatri S. Jawanjal[1]*, Jogeshwari S. Burungale[2], Rajeshwar R. Hingane[3], Urmila M. Tiwasker[1], Pushpa G. Vaidya[1], Snehal N. Uike[4], Aishwarya M. Hiwase[5]

[1]*Shri Gurudatta Shikshan Prasarak Sanstha Institute of Pharmacy, Kaulkhed, Akola*
[2]*PRMMS Anuradha College of Pharmacy Chikhli*
[3]*Lokmanya Tilak Institute of pharmaceutical Sciences, Pune*
[4]*R. Pote Patil College Of Pharmacy Amravati*
[5]*Pataldhamal Wadhwani College of Pharmacy , Yavatmal*

ABSTRACT

Animal-based research has historically served as the cornerstone of biomedical science, providing crucial insights into human physiology, pharmacology, toxicology, and disease mechanisms The use of *in vivo* models particularly rodents and other laboratory animals has enabled the development of vaccines, therapeutics, and diagnostic tools that have significantly advanced healthcare. However, despite their contributions, these models face growing ethical, translational, and practical challenges. Ethical concerns regarding animal welfare, coupled with interspecies biological variations, often limit the direct applicability of animal data to human system. Notably, only a small percentage of drug candidates showing promise in animal studies achieve success in human clinical trials, underscoring the predictive limitations of traditional animal testing. Driven by these challenges and recent technological progress, biomedical research is experiencing a paradigm shift "beyond the cage" toward the adoption of computational (*in silico*) models that simulate biological processes digitally. These models integrate artificial intelligence (AI), bioinformatics, and systems biology to predict drug interactions, assess toxicity, and model disease mechanisms with high precision. The integration of *in silico, in vitro* and *in vivo* methodologies is emerging as a synergistic approach, combining the holistic biological perspective of live-animal studies with the predictive power and ethical advantages of computational systems. This review explores recent advances in *in vivo, in vitro* and *in silico* modeling, evaluates their respective strengths and limitations, and highlights the global regulatory and ethical movements supporting humane and human-relevant research. Ultimately, this transition signifies a critical evolution toward efficient, ethical, and translationally relevant science in the modern biomedical landscape.

Keywords: In Vivo Models; In Silico Modeling; Artificial Intelligence; Drug Discovery; Translational Research.

INTRODUCTION

Animal testing has long been essential in biomedical and pharmaceutical research, contributing to major medical advances such as vaccines and life-saving drugs. However, ethical concerns, high costs, lengthy procedures, and differences between animal and human biology have raised questions about its effectiveness and reliability.As a result, researchers are increasingly adopting alternative approaches.[1] In vitro methods use human cells, tissues, organoids, and organ-on-a-chip technologies to study biological processes in controlled laboratory settings. In silico methods employ computer simulations, artificial intelligence, and mathematical models to predict drug responses and disease behavior.[2]Today, in vivo, in vitro, and in silico approaches are often combined to improve research accuracy, reduce animal use, and accelerate medical innovation. Regulatory agencies worldwide support these New Approach Methodologies (NAMs), promoting a more ethical, efficient, and human-relevant future for biomedical research.[3]

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

In Vivo:

Methodologies in Modern Research:

In vivo research uses living animals to study drug safety, effectiveness, metabolism, and complex biological interactions in a whole-body system. It plays a key role in drug development and regulatory testing. However, ethical concerns, high costs, long study durations, and differences between animal and human responses limit its effectiveness. Therefore, modern research increasingly combines in vivo methods with in vitro and in silico approaches to improve accuracy, efficiency, and ethical standards.[4]

In Vitro Models:

In Vitro Platforms in Modern Biomedicine:

In vitro models are laboratory-based systems that use isolated cells, tissues, or microorganisms to study biological processes outside a living organism. Advances in cell biology, tissue engineering, and microfluidics have made these models more realistic and capable of mimicking human organ functions. They serve as an important link between animal studies and computer-based simulations, offering better control over experimental conditions and providing valuable tools for drug development, toxicology, regenerative medicine, and personalized healthcare.[5]

Human-Derived Organoids:

Human-derived organoids are three-dimensional mini-organ models created from stem cells that can mimic the structure and function of real human organs. They are used to study diseases, test medicines, and support regenerative medicine research. Example include brain organoids for investigating neurological disorders and liver organoids for assessing drug-related liver damage. Since organoids retain the genetic characteristics of the donor, they are valuable tools for personalized medicine and individualized drug testing.[6]

Organ-on-a-Chip Systems:

Organ-on-a-Chip (OoC) systems are small devices that combine engineering and biology to imitate the structure and function of human organs. They use living cells in tiny channels to recreate natural conditions such as fluid flow and mechanical movements. Examples include lung chips that simulate breathing and gut chips that mimic intestinal motion. Advanced versions connect multiple organ models to study drug effects, toxicity, and interactions between organs, providing a more realistic and efficient alternative for biomedical research.[7]

Silico Models:

The New Computational Frontier:

In silico models use computer-based simulations and mathematical methods to study biological systems and predict outcomes. Unlike animal-based (in vivo) or laboratory-based (in vitro) experiments, these approaches are performed entirely on computers. Advances in artificial intelligence, bioinformatics, and data analysis have made in silico methods an important part of biomedical research, enabling faster, scalable, and more ethical studies of drug interactions, diseases, and biological processes.[8]

Application of In-silico Modules:[9]

1. Data discovery and toxicology
2. System biology
3. Virtual organs and digital patients
4. Predictive Toxicology and Artificial intellangence

Integrating In Vivo, In Vitro, and In Silico Strategies:

The future of biomedical research lies in combining in vivo (animal-based), in vitro (cell-based), and in silico (computer-based) methods rather than relying on a single approach. This integrated strategy improves the accuracy of research, reduces dependence on animal testing, and supports ethical principles such as the 3Rs (Reduction, Refinement, and Replacement). By using multiple tools together, scientists can better predict human responses, making research more efficient, cost-effective, and relevant to patient care.[10]

Hybrid Modeling:

Bridging Empirical and Computational Evidence:

Hybrid modeling combines data from animal studies with computer-based simulations to improve research accuracy and efficiency. It uses experimental findings, such as drug effects and toxicity data, together with computational models to predict how treatments may behave in humans. This approach helps optimize drug dosing, enhance safety assessments, reduce unnecessary animal testing, and continuously improve model performance through feedback from new experimental results. Overall, hybrid modeling supports more reliable and ethical biomedical research.[11]

AI-Assisted Experimental Design and Optimization:

Artificial intelligence (AI) and machine learning (ML) are improving research design by helping scientists choose the most effective experiments while reducing unnecessary testing. These technologies analyzed large datasets to predict useful study methods, optimize testing conditions, and minimize redundant animal experiments, supporting ethical research practices. AI also helps organize and combine data from different sources, improving efficiency, reproducibility, and the reliability of computational models.[12]

In Vitro–In Silico Coupling: A Multiscale Strategy:

The combination of in vitro experiments and in silico models enhances the ability to predict biological responses accurately. Advanced laboratory systems, such as organoids and organ-on-a-chip platforms, provide detailed cellular data that can be integrated into computational models to simulate drug metabolism, disease processes, and toxicity. This two-way interaction improves model accuracy, supports validation, and helps researchers predict the effects of substances across multiple biological levels, reducing the need for extensive experimental testing.[13]

Data-Driven Refinement and Feedback Loops:

Modern biomedical research relies on integrating data from in vivo, in vitro, and in silico studies to improve prediction accuracy and reproducibility. Computational models and experimental data continuously inform and refine each other, creating a cycle that strengthens scientific findings. This approach supports the development of predictive toxicity frameworks and machine learning models, while large biological databases enable ongoing improvement, transparency, and reliability in biomedical research.[14]

Applications Across Biomedical Fields:

The combined use of in vivo, in vitro, and in silico methods has improved research in drug development, toxicology, disease model and precision medicine. Computational tools help identify promising compounds, laboratory models verify biological effects, and animal studies assess overall safety and effectiveness. Together, these approaches enhance predictive accuracy, support personalized treatments, reduce animal testing, and provide a more comprehensive understanding of complex diseases.[15]

Ethical and Regulatory Implications

The integrative approach aligns seamlessly with ethical imperatives and global regulatory frameworks advocating humane science. International organizations such as the OECD, FDA, EMA, and EU REACH now endorse the use of New Approach Methodologies (NAMs), which combine computational, in vitro, and limited in vivo testing to ensure human safety while minimizing animal use. Ethical oversight bodies are increasingly recognizing integrated modeling as a legitimate and reliable strategy for regulatory decision-making, especially when supported by robust validation and cross-model correlation. This trend reflects a broader shift toward evidence-based, transparent, and compassionate research practices that harmonize innovation with responsibility.[16]

Regulatory and Ethical Perspectives:

Biomedical research is increasingly adopting ethical and innovative approaches that reduce reliance on animal testing. New Approach Methodologies (NAMs), which combine in vitro, in silico, and selective in vivo methods, provide reliable and human-relevant data while supporting animal welfare. Regulatory agencies and international organizations

are promoting the validation and acceptance of these alternatives, encouraging research practices that balance scientific quality, regulatory requirements, and ethical responsibility.[17]

The 3Rs Principle: Foundation of Humane Science:

The 3Rs principle—Replacement, Reduction, and Refinement forms the ethical foundation of modern animal research. Replacement promotes the use of alternatives such as organoids, organ-on-chip systems, and computer-based models instead of animals whenever possible. Reduction focuses on using the minimum number of animals needed to obtain reliable scientific results, while Refinement aims to minimize pain, stress, and suffering through improved care and experimental practices. Together, these principles enhance both animal welfare and research quality and are widely incorporated into international regulations and guidelines[18].

Ethical Governance, Transparency, and Global Policy Alignment:

Modern regulatory systems emphasize transparency, data sharing, and reproducibility as integral components of ethical science. Open-access databases and cross-institutional collaborations such as the OECD's QSAR Toolbox, EURL-ECVAM Knowledge Base, and ToxCast facilitate global sharing of validated non-animal data. These initiatives reduce redundant testing, improve reproducibility, and promote the regulatory acceptance of NAMs. Regulatory bodies are also investing in open science policies, mandating that researchers make preclinical and clinical data publicly accessible. This ensures accountability, allows for independent validation, and strengthens public trust in scientific institutions. Ethical oversight committees, including Institutional Animal Care and Use Committees (IACUCs) and equivalent bodies in Europe and Asia, are increasingly adopting AI-driven decision-support systems to assess study justification, refine experimental designs, and ensure compliance with the 3Rs. Such governance mechanisms ensure that all scientific advancement aligns with humanitarian values and global sustainability goals [19].

Organs-on-Chips and Microphysiological Systems:

Organs-on-chips (OoCs) are advanced microengineered devices that use human cells and microfluidic technology to mimic the structure and function of human organs. They provide a realistic platform for studying drug effects, toxicity, and disease processes. Microphysiological systems (MPS) further connect multiple organ models to simulate interactions between different organs within the body. These technologies have demonstrated strong potential for predicting human responses to drugs and reducing relicence on animal testing. Their integration with computational models and real-time data analysis enhances drug development, safety assessment, and personalized medicine.[20]

Human Stem Cell–Derived Models and Organoids:

Human pluripotent stem cells (hPSCs), including induced pluripotent stem cells (iPSCs), have enabled the development of organoids—three-dimensional tissue models that closely resemble human organs. These organoids replicate important structural and functional characteristics of tissues such as the brain, liver, intestine, and kidney, making them valuable tools for studying diseases, infections, and drug responses. They provide patient-specific insights into conditions like cancer and genetic disorders. When integrated with computational models, organoids improve the prediction of drug efficacy and toxicity using real human biological data. Additionally, organoid biobanks support large-scale precision medicine research by capturing genetic and biological diversity across populations.[21]

3D Bioprinting: Engineering the Human Microenvironment:

3D bioprinting is an advanced technology that creates living tissues and organ-like structures by precisely depositing cells and biomaterials layer by layer. It enables the development of realistic tissue models that closely mimic human organ structure and function, making it useful for drug testing, toxicity assessment, and regenerative medicine. Recent progress in vascularized bioprinting has improved the ability to produce tissues with efficient nutrient and oxygen supply. When combined with computational

modeling, 3D bioprinting enhances the prediction of tissue responses and helps optimize experimental design. This integration supports the future development of personalized tissues and organs for research and potential therapeutic applications.[22]

Artificial Intelligence and Machine Learning in Predictive Medicine:

Artificial intelligence (AI) and machine learning (ML) are transforming biomedical research by analyzing large biological and clinical datasets to improve disease prediction, drug discovery, and treatment optimization. These technologies help identify potential drug candidates, predict adverse drug reactions, and support personalized virtual clinical trials, reducing the need for extensive animal testing. A major advancement in this field is the development of human digital twins—virtual models of individual patients created using genetic, clinical, imaging, and sensor data. Digital twins enable real-time monitoring, prediction of disease progression, and personalized treatment planning, offering significant potential for precision medicine and improved healthcare outcomes.[23]

Human Digital Twins: Toward Personalized Simulation

Perhaps the most futuristic advancement is the concept of human digital twins — virtual replicas of individual patients that integrate data from genomics, proteomics, medical imaging, and wearable sensors to simulate disease progression and therapeutic response. Digital twins can continuously update with new patient data, enabling real-time monitoring, risk assessment, and personalized treatment planning. For instance, cardiac digital twins can model electrophysiological activity to predict arrhythmia risk, while oncology twins simulate tumor dynamics to optimize targeted therapies. This technology represents the ultimate integration of in silico, in vitro, and clinical data, bridging predictive modeling with personalized healthcare. The European Commission's Digital Twin Initiative and projects like Sim Cardio Test and Virtu Heart are pioneering these efforts, with early results demonstrating high predictive validity for cardiovascular and metabolic diseases. [24]

Toward a Human-Relevant, Ethical, and Efficient Research Ecosystem

The long-term vision for biomedical science is to establish a fully integrated, human-centered research ecosystem. By combining the biological realism of in vitro systems, the systemic insight of in vivo validation, and the predictive intelligence of in silico models, researchers can achieve a paradigm shift in scientific discovery. This evolution will not only reduce animal testing but also enhance translational relevance, leading to safer drugs, more effective treatments, and greater public trust in science. As interdisciplinary collaboration deepens and regulatory frameworks evolve, the next decade promises a revolution in how we conceptualize and conduct biomedical research — one that transcends ethical, technical, and biological boundaries. [25]

Future Prospects:

The future of biomedical science is being reshaped by an unprecedented convergence of biotechnology, computational intelligence, and ethical innovation. The traditional divide between in vivo, in vitro, and in silico research is rapidly dissolving, giving rise to integrated systems that simulate human biology with exceptional fidelity. Emerging technologies such as organs-on-chips, stem cell–derived human organoids, 3D bioprinting, artificial intelligence (AI), and human digital twins are revolutionizing predictive modeling, drug discovery, and toxicology. Together, these advancements promise a research ecosystem that is not only more human-relevant but also more efficient, ethical, and sustainable.

CONCLUSION

"Beyond the Cage" represents a modern approach to biomedical research that integrates animal studies, advanced cell-based models, and computational technologies to improve scientific accuracy while reducing animal use. By combining ethical practices with innovative tools such as AI and predictive modeling, this approach enhances drug development, disease understanding, and personalized medicine. It supports the principles of the 3Rs and promotes a future where scientific progress and ethical responsibility work together to advance human health.

REFERENCES

1. Festing, M.F.W., & Wilkinson, R. (2007). The ethics of animal research. EMBO Reports, 8(6), 526–530.
2. Bailey, J., Thew, M., & Balls, M. (2019). An analysis of the use of animal models in predicting human toxicology and drug safety. Alternatives to Laboratory Animals, 47(1), 5–22
3. Franco, N.H. (2013). Animal experiments in biomedical research: A historical perspective. Animals, 3(1), 238–273.
4. Pound, P., & Ritskes-Hoitinga, M. (2018). Issues of external validity in preclinical animal research. BMJ Open Science, 2(1).
5. Franco, N.H., Olsson, I.A.S., & Hansen, A.K. (2018). Ethics of animal experimentation: Historical and conceptual overview. Lab Animal, 47(11), 289–299.
6. van der Worp, H.B. et al. (2010). Can animal models predict human outcomes? Nature Reviews Neuroscience, 11(6), 466–470.
7. Hay, M. et al. (2014). Clinical development success rates for investigational drugs. Nature Biotechnology, 32(1), 40–51.
8. Leist, M., & Hartung, T. (2013). In vitro methods and human predictive toxicology. Chemical Research in Toxicology, 26(4), 469–479.
9. Hartung, T. (2009). Toxicology for the twenty-first century. Nature, 460(7252), 208–212.
10. Pampaloni, F., Reynaud, E.G., & Stelzer, E.H.K. (2007). The third dimension bridges the gap between cell culture and live tissue. Nature Reviews Molecular Cell Biology, 8(10), 839–845.
11. Bhatia, S.N., & Ingber, D.E. (2014). Microfluidic organs-on-chips. Nature Biotechnology, 32(8), 760–772.
12. Clevers, H. (2016). Modeling development and disease with organoids. Cell, 165(7), 1586– 1597.
13. Huh, D. et al. (2010). Reconstituting organ-level lung functions on a chip. Science, 328(5986), 1662–1668.
14. Lancaster, M.A. & Knoblich, J.A. (2014). Organogenesis in a dish: Modeling development and disease using organoid technologies. Science, 345(6194).
15. Ekins, S. et al. (2007). In silico pharmacology for drug discovery: Methods for virtual ligand screening and profiling. British Journal of Pharmacology, 152(1), 9–20.
16. Raies, A.B., & Bajic, V.B. (2016). In silico toxicology: Computational methods for the prediction of chemical toxicity. Wiley Interdisciplinary Reviews: Computational Molecular Science, 6(2), 147–172.
17. Helma, C. et al. (2018). From structure–activity relationships toward deep learning and mechanistic models in toxicology. Computational Toxicology, 6, 61–67.
18. Viceconti, M., & Hunter, P. (2016). The Virtual Physiological Human: Ten years after. Annual Review of Biomedical Engineering, 18, 103–123.
19. Corral-Acero, J. et al. (2020). The “Digital Twin” to enable the vision of precision cardiology. European Heart Journal, 41(48), 4556–4564.
20. Marx, U. et al. (2020). Biology-inspired microphysiological systems to advance patient benefit and animal welfare. ALTEX, 37(3), 364–394.
21. Russell, W.M.S., & Burch, R.L. (1959). The Principles of Humane Experimental Technique. Methuen, London.
22. OECD. (2021). Guidance Document on Good In Vitro Method Practices (GIVIMP). OECD Publishing.
23. Escher, S.E. et al. (2020). Integrating in silico, in vitro and in vivo approaches for next- generation risk assessment. Archives of Toxicology, 94, 3899–3917.
24. Viceconti, M., & Hunter, P. (2016). The Virtual Physiological Human: Ten years after. Annual Review of Biomedical Engineering, 18, 103–123.
25. Helma, C. (2018). Artificial intelligence in toxicology—From QSARs to deep learning. Frontiers in Pharmacology, 9, 1090.

HOW TO CITE: Gayatri S. Jawanjal[1]*, Jogeshwari S. Burungale[2], Rajeshwar R. Hingane[1], Urmila M. Tiwasker[1], Pushpa G. Vaidya[1], Snehal N. Uike[1], Aishwarya M. Hiwase[1], Beyond The Cage: Advancing Science In In-Vivo, In-Vitro & In-Silico Models, Int. J. Sci. R. Tech., 2026, 3 (6), 11-16. https://doi.org/10.5281/zenodo.20483085