

Review Articles



Integrating the 3Rs into Modern Biomedical Research: Ethical Innovations and Multidisciplinary Strategies

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ARTICLE INFO

Article history:

Received 12 March 2026

Accepted 22 March 2026

Keywords:

3Rs Principles;

Biomedical Research Ethics;

Alternative Research Models;

Animal Welfare;

Translational Research

ABSTRACT

The ethical use of animals in biomedical research has been shaped by the guiding framework of the 3Rs—Replacement, Reduction and Refinement. With rapid advancements in biotechnology, computational sciences and translational medicine, the practical integration of these principles has evolved beyond regulatory compliance toward innovation-driven ethical research. This review examines emerging multidisciplinary strategies that operationalize the 3Rs across modern biomedical research pipelines. Replacement approaches increasingly employ human-relevant alternatives such as advanced in vitro models, organ-on-chip platforms, computational simulations, and artificial intelligence-assisted predictive models, reducing reliance on traditional animal experimentation. Reduction strategies emphasize rigorous experimental design, improved statistical methodologies, data-sharing initiatives, and high-resolution imaging technologies that maximize data output while minimizing animal use. Refinement focuses on improving animal welfare through enhanced housing conditions, minimally invasive monitoring technologies, precision analgesia and welfare-based humane endpoints. The integration of these principles is strengthened by collaboration among biologists, veterinarians, engineers, bioinformaticians, and ethicists. Furthermore, regulatory frameworks and funding agencies increasingly promote the adoption of innovative alternative methodologies aligned with the 3Rs. By embedding ethical responsibility within scientific practice, the multidisciplinary implementation of the 3Rs contributes to improved research reproducibility, enhanced translational relevance and greater societal acceptance of biomedical research. Ultimately, advancing ethical innovations in research design supports the transition toward more predictive, human-relevant models while maintaining high standards of animal welfare.

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1. Introduction

The ethical use of animals in biomedical research has undergone substantial conceptual and methodological evolution over the past several decades. Animal experimentation historically played a central role in understanding disease mechanisms, evaluating pharmacological interventions, and ensuring therapeutic safety. However, increasing societal concern regarding animal welfare and scientific debates about the translational validity of animal models led to the emergence of structured ethical frameworks ^[1]. One of the most influential among these is the 3Rs principle—Replacement, Reduction, and Refinement—proposed by Russell and Burch in 1959 as a strategy to promote humane and scientifically responsible research practices ^[2]. Initially, the implementation of the 3Rs principle was largely compliance-oriented, focusing on minimizing

unnecessary animal use within experimental protocols. Over time, technological advancements in biotechnology, computational sciences, and tissue engineering have transformed the operational scope of the 3Rs from regulatory obligation to innovation-driven research strategy ^[3]. Contemporary biomedical research increasingly integrates human-relevant experimental platforms such as organoids, micro-physiological systems, and artificial intelligence-based predictive models, thereby enhancing translational relevance while improving ethical accountability. Despite these advancements, significant challenges remain in the global implementation of the 3Rs framework ^[4]. Variability in regulatory acceptance, insufficient standardization of alternative models, and disparities in technological access between developed and developing countries continue to influence the practical adoption of humane research strategies. Therefore, a comprehensive multidisciplinary assessment of emerging innovations, implementation gaps, and future prospects is

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DOI: 10.64904/fpm2026.025



necessary to strengthen the scientific and ethical foundations of modern biomedical research [5].

2. Methodology for literature search

A systematic trend-based narrative review methodology was employed to analyze recent developments in the implementation of the 3Rs within biomedical research [6].

2.1. Data sources

Scientific literature relevant to the study was retrieved from major academic databases, including PubMed, Scopus, Web of Science, and ScienceDirect.

2.2. Search strategy

Relevant literature was identified using combinations of keywords, including “3Rs principles in biomedical research,” “replacement, reduction, and refinement,” “alternative methods to animal testing,” “organ-on-chip technologies,” and “ethical frameworks in biomedical experimentation.”

2.3. Inclusion criteria

Studies were included if they addressed ethical or methodological aspects of animal research, reported innovations related to replacement, reduction, or refinement, presented experimental, technological, or policy-oriented approaches supporting the 3Rs, and were published in recent years to capture emerging research trends.

2.4. Exclusion criteria

Studies were excluded if they consisted of non-scientific opinion articles, were published in languages other than English, or were unrelated to biomedical or translational research.

2.5. Data analysis

Selected literature was thematically categorized according to the three core principles of the 3Rs. Additional emphasis was placed on interdisciplinary strategies, technological innovations, and ethical frameworks supporting modern biomedical research practices.

3. Conceptual framework of the 3Rs in biomedical research

The conceptual framework of the 3Rs in biomedical research represents an integrated ethical-scientific approach designed to harmonize experimental advancement with humane responsibility. Rather than functioning as isolated procedural guidelines, the principles of Replacement, Reduction, and Refinement collectively form a dynamic decision-making model that influences research design, methodological selection, and translational interpretation across the biomedical research continuum. Replacement constitutes the strategic adoption of non-animal methodologies or lower-sentience biological systems whenever scientifically feasible. This includes the use of human-derived in vitro platforms, organoid technologies, computational simulations, and predictive artificial intelligence models that enhance human relevance while reducing dependence on traditional animal

experimentation. Reduction involves the optimization of experimental planning to ensure that the minimum number of animals is used to obtain statistically robust and reproducible outcomes. Advanced biostatistical tools, longitudinal experimental designs, shared data infrastructures, and high-resolution imaging technologies contribute to maximizing information yield per experimental subject. Refinement focuses on the continuous improvement of experimental procedures, environmental conditions, and welfare monitoring systems to minimize pain, distress, and physiological burden experienced by animals. Strategies such as precision analgesia, environmental enrichment, non-invasive monitoring, and predefined humane endpoints not only improve animal well-being but also enhance experimental reliability by reducing stress-related variability. Within this conceptual framework, the 3Rs operate synergistically to strengthen scientific rigor, ethical accountability, and societal acceptance of biomedical research. Their integrated implementation supports the transition toward more predictive and human-relevant research models while maintaining high standards of animal welfare and methodological validity [7].

4. Replacement strategies: emerging alternative research models

Replacement strategies represent a central pillar of the 3Rs framework, focusing on the progressive substitution of conventional animal experimentation with scientifically valid alternative approaches. The objective of replacement is not merely to eliminate animal use, but to adopt experimental systems that provide equal or superior mechanistic understanding, predictive accuracy, and translational relevance for human health research. Recent advances in biotechnology and computational sciences have accelerated the development of human-relevant research platforms capable of modelling complex physiological and pathological processes. These include sophisticated in vitro models such as three-dimensional cell culture systems, stem-cell-derived organoids and tissue-engineered constructs that replicate structural and functional characteristics of human organs. In parallel, micro physiological systems and organ-on-chip technologies integrate microfluidic engineering with living tissues to simulate dynamic biological interactions under controlled experimental conditions. Furthermore, the growing application of in silico methodologies, including systems biology modelling, machine learning-based toxicity prediction, and virtual drug screening, enables early-stage hypothesis testing and risk assessment without reliance on animal models. These approaches contribute to improved research efficiency, reduced experimental costs, and enhanced translational predictability. However, despite their significant potential, challenges related to validation, standardization, and regulatory acceptance continue to influence the widespread implementation of replacement strategies. Collectively, emerging alternative research models are reshaping the landscape of biomedical investigation by promoting ethically responsible innovation while supporting the transition toward more human-centric experimental paradigms [8].

4.1. Advanced In Vitro models

Advanced in vitro experimental models have emerged as pivotal tools in replacement strategies by enabling mechanistic investigations of biological processes within controlled and human-relevant environments. Cell-based systems facilitate the exploration of molecular signaling pathways, disease pathophysiology, and pharmacological responses using human-derived

tissues, thereby improving the translational validity of preclinical findings. Recent technological progress has led to the development of sophisticated three-dimensional culture platforms, including scaffold-based tissue-engineered constructs and stem-cell-derived multicellular models that better reproduce the structural organization and functional dynamics of native human tissues. These systems allow for enhanced cell–cell and cell–matrix interactions, improved metabolic activity, and more accurate modelling of disease phenotypes compared with traditional two-dimensional cultures. In addition, integration with microfluidic technologies and high-content imaging approaches has enabled dynamic assessment of drug responses, toxicity mechanisms, and tissue regeneration processes over time. Such advancements contribute to increased experimental reproducibility, reduction in interspecies variability, and improved prediction of clinical outcomes. Nevertheless, challenges related to long-term tissue viability, standardization of culture conditions, and limited systemic interaction remain important considerations for the broader application of advanced *in vitro* models in biomedical research ^[9].

4.2. Organoids and micro-physiological systems

Organoid technologies have emerged as a transformative component of replacement strategies by enabling the generation of three-dimensional, stem-cell-derived tissue constructs that recapitulate key structural and functional features of native human organs. These self-organizing biological systems provide physiologically relevant platforms for investigating developmental biology, disease mechanisms, and pharmacological responses under controlled laboratory conditions. Compared with conventional cell culture models, organoids demonstrate improved cellular heterogeneity, spatial organization, and functional maturation, thereby enhancing the predictive validity of preclinical research. Complementing organoid approaches, micro-physiological systems—commonly referred to as organ-on-chip technologies—integrate living cells with microfluidic engineering to simulate dynamic biomechanical and biochemical interactions observed *in vivo*. These platforms enable precise regulation of parameters such as fluid flow, oxygen gradients, and mechanical stress, allowing researchers to model complex physiological processes including drug absorption, metabolism, and tissue-level toxicity. Multi-organ chip configurations further facilitate the investigation of systemic interactions and inter-organ communication, which are critical for improving translational interpretation of experimental findings. Despite their considerable scientific promise, several limitations continue to influence the widespread adoption of organoids and micro-physiological systems. Challenges related to reproducibility, scalability, long-term functional stability, and regulatory validation remain areas of active investigation. Addressing these constraints through standardized protocols, interdisciplinary collaboration, and integration with computational modelling is essential for maximizing the potential of these advanced human-relevant experimental platforms in modern biomedical research ^[10].

4.3. Computational modeling and artificial intelligence

Computational modelling and artificial intelligence (AI) have become integral components of modern replacement strategies by enabling the simulation of complex biological processes and the prediction of pharmacological and toxicological responses without direct reliance on animal experimentation. These approaches support hypothesis generation, target identification, and risk assessment during the early stages of biomedical research, thereby improving experimental efficiency and ethical

responsibility. Advanced systems biology models facilitate the integration of multi-omics data to construct mechanistic representations of cellular networks and disease pathways. In parallel, machine learning and deep learning algorithms are increasingly applied for quantitative structure–activity relationship (QSAR) analysis, toxicity prediction, and pharmacokinetic–pharmacodynamic (PK–PD) modelling. Virtual drug screening platforms and digital compound libraries further enable high-throughput evaluation of therapeutic candidates, significantly reducing the need for exploratory animal studies. The incorporation of AI-driven predictive analytics also contributes to the development of emerging concepts such as digital twins and personalized disease modelling, which hold potential for enhancing translational accuracy in preclinical research. However, the broader implementation of computational methodologies remains influenced by challenges related to data quality, model validation, algorithm transparency, and regulatory acceptance. Continued integration of computational tools with experimental human-relevant platforms is therefore essential to establish robust and ethically sustainable research paradigms in biomedical science ^[11].

5. Reduction strategies in experimental design

Reduction strategies in biomedical research aim to minimize the number of animals used in experimental studies while ensuring that scientific validity, statistical reliability, and reproducibility of findings are not compromised. Rather than simply decreasing sample size, the principle of reduction emphasizes the optimization of experimental design and data utilization to maximize the information obtained from each experimental subject.

Rigorous biostatistical planning plays a central role in achieving reduction objectives. Techniques such as power analysis, adaptive experimental designs, factorial study models, and Bayesian statistical approaches enable researchers to determine the minimum number of animals required to generate meaningful and interpretable results. In addition, improved randomization, blinding procedures, and standardized experimental protocols contribute to reducing variability and enhancing data quality. Technological advancements further support reduction strategies by enabling longitudinal assessment within the same experimental animal. High-resolution imaging modalities, including magnetic resonance imaging, positron emission tomography, and optical imaging techniques, allow repeated non-invasive monitoring of disease progression, therapeutic response, and physiological changes over time. Such approaches significantly decrease the need for multiple experimental cohorts. Furthermore, open science initiatives and collaborative data-sharing platforms facilitate the reuse of existing experimental datasets, thereby preventing unnecessary duplication of animal studies across research institutions. The integration of these methodological and technological innovations enhances research efficiency, reduces experimental costs, and strengthens the ethical and scientific justification for animal use in biomedical investigations ^[12].

5.1. Advanced statistical design

Advanced statistical design constitutes a critical component of reduction strategies by enabling researchers to determine the optimal sample size required to achieve scientifically valid and reproducible outcomes. Robust statistical planning ensures that experimental studies are adequately powered to detect meaningful biological effects while avoiding unnecessary use of experimental animals. Key methodological approaches include prospective power analysis for sample size estimation, adaptive and

sequential experimental designs that allow interim data evaluation, and factorial study models that enable simultaneous assessment of multiple variables within a single experimental framework. In addition, Bayesian statistical methods provide flexible probabilistic inference by integrating prior knowledge with emerging experimental data, thereby supporting more efficient decision-making during study progression. The adoption of rigorous randomization procedures, blinding techniques, and standardized outcome measures further contributes to minimizing experimental bias and variability. Collectively, these advanced statistical strategies enhance data quality, improve experimental efficiency, and strengthen the ethical justification for animal use by ensuring that each study generates maximal scientific value with minimal resource utilization ^[13].

5.2. Data sharing and collaborative research

Data sharing and collaborative research frameworks play a pivotal role in supporting reduction strategies by enabling the efficient utilization of existing experimental evidence and preventing unnecessary duplication of animal studies. The growing adoption of open science initiatives has facilitated the establishment of centralized data repositories, institutional research networks, and interdisciplinary consortia that promote transparent dissemination and reuse of experimental findings. Access to shared datasets allows researchers to perform secondary analyses, meta-analytical evaluations, and integrative modelling approaches without the need to initiate new animal experiments. International databases, standardized reporting guidelines, and harmonized data management practices further contribute to improving research reproducibility and methodological consistency across institutions. In addition, collaborative multicentre studies enable the pooling of resources and expertise, thereby enhancing statistical power and reducing the total number of animals required at individual research sites. Despite these advantages, effective implementation of data-sharing practices remains influenced by challenges related to data standardization, intellectual property considerations, and variability in regulatory frameworks. Strengthening digital infrastructure, promoting ethical data governance policies, and encouraging interdisciplinary collaboration are therefore essential for maximizing the scientific and ethical benefits of shared research ecosystems in biomedical science ^[14].

5.3. Imaging technologies

Non-invasive imaging technologies represent a major methodological advancement in reduction strategies by enabling longitudinal assessment of disease progression, therapeutic response, and physiological changes within the same experimental animal. This approach allows repeated measurements over time, thereby reducing inter-animal variability and minimizing the need for multiple experimental cohorts. Advanced imaging modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and optical imaging techniques facilitate high-resolution visualization of structural, functional, and molecular processes in vivo. Functional imaging approaches, including contrast-enhanced MRI and radiotracer-based PET imaging, enable real-time evaluation of tissue perfusion, metabolic activity, and receptor-specific drug distribution. These capabilities are particularly valuable in fields such as oncology, neuroscience, and cardiovascular research, where dynamic monitoring of pathological processes is essential for translational interpretation. Furthermore, the integration of imaging platforms with quantitative image analysis, automated segmentation algorithms, and

artificial intelligence-assisted data interpretation enhances experimental precision and reproducibility. By maximizing the informational output obtained from individual animals, non-invasive imaging contributes to improved study efficiency and ethical justification of animal use. Nevertheless, considerations related to equipment cost, technical expertise, and potential imaging-associated stress must be addressed to ensure optimal implementation of these technologies in biomedical research settings ^[15].

6. Refinement strategies for animal welfare

Refinement strategies focus on the continuous improvement of experimental procedures, housing conditions, and welfare monitoring practices in order to minimize pain, distress, and physiological burden experienced by animals during biomedical research. Beyond ethical considerations, refinement also contributes to enhanced scientific reliability by reducing stress-induced variability and improving the overall quality of experimental data. Key refinement approaches include the adoption of minimally invasive or non-invasive techniques, optimization of anaesthetic and analgesic protocols and implementation of welfare-oriented experimental endpoints. Precision analgesia strategies tailored to species, experimental models, and study duration are increasingly emphasized to ensure effective pain management without interfering with research outcomes. In addition, predefined humane endpoints—such as early clinical or behavioural indicators of disease severity—allow timely intervention or termination of experiments to prevent unnecessary suffering. Environmental refinement measures, including enriched housing conditions, appropriate social grouping, and controlled microclimatic parameters, play a critical role in maintaining psychological well-being and physiological stability in laboratory animals. Technological innovations such as automated behavioural tracking systems, wearable biosensors, and remote health monitoring platforms further enable continuous assessment of animal welfare while minimizing handling-related stress. Collectively, the systematic implementation of refinement strategies supports the development of ethically responsible research methodologies while simultaneously enhancing experimental reproducibility, translational relevance, and societal acceptance of animal-based biomedical investigations ^[16].

6.1. Improved housing and environmental enrichment

Appropriate housing conditions are fundamental for maintaining physiological stability and behavioural normalcy in laboratory animals. Environmental enrichment strategies, such as provision of nesting materials, shelters, social interaction, and species-specific stimulation, contribute to improved psychological well-being and adaptive behaviour. Optimized microenvironmental parameters including temperature, humidity, and lighting cycles further support health and experimental consistency. Such welfare-oriented housing practices reduce stress-induced variability and enhance the reliability and reproducibility of research outcomes. Consequently, structured environmental management is considered an essential component of refinement-based ethical research design ^[17].

6.2. Non-Invasive monitoring technologies

Technological innovations have enabled continuous and real-time monitoring of animal health without the need for invasive handling or procedural stress. Tools such as wearable biosensors, automated behavioural tracking systems, and wireless telemetry devices facilitate the

assessment of physiological parameters including activity patterns, heart rate, body temperature, and circadian rhythm. These approaches allow early detection of disease progression and welfare compromise while supporting longitudinal data collection within the same experimental subject. By minimizing handling-related disturbances, non-invasive monitoring improves both animal well-being and data reliability. Consequently, such technologies represent a significant advancement in refinement-oriented experimental design [18].

6.3. Precision analgesia and humane endpoints

Modern veterinary practices emphasize effective pain management and early humane endpoints to minimize animal distress. The integration of the

3Rs principles-Replacement, Reduction and Refinement-plays a crucial role in promoting ethical and scientifically reliable biomedical research. These principles guide researchers in minimizing animal use while ensuring high-quality experimental outcomes. Figure 1 illustrates the conceptual framework of the 3Rs, highlighting how replacement strategies encourage the adoption of alternative research models, reduction focuses on optimized experimental design and data sharing to limit animal use and refinement emphasizes improved animal welfare through enhanced housing, humane endpoints and non-invasive technologies. Together, these interconnected strategies enhance data quality, reduce experimental variability and support responsible and humane research practices [19].

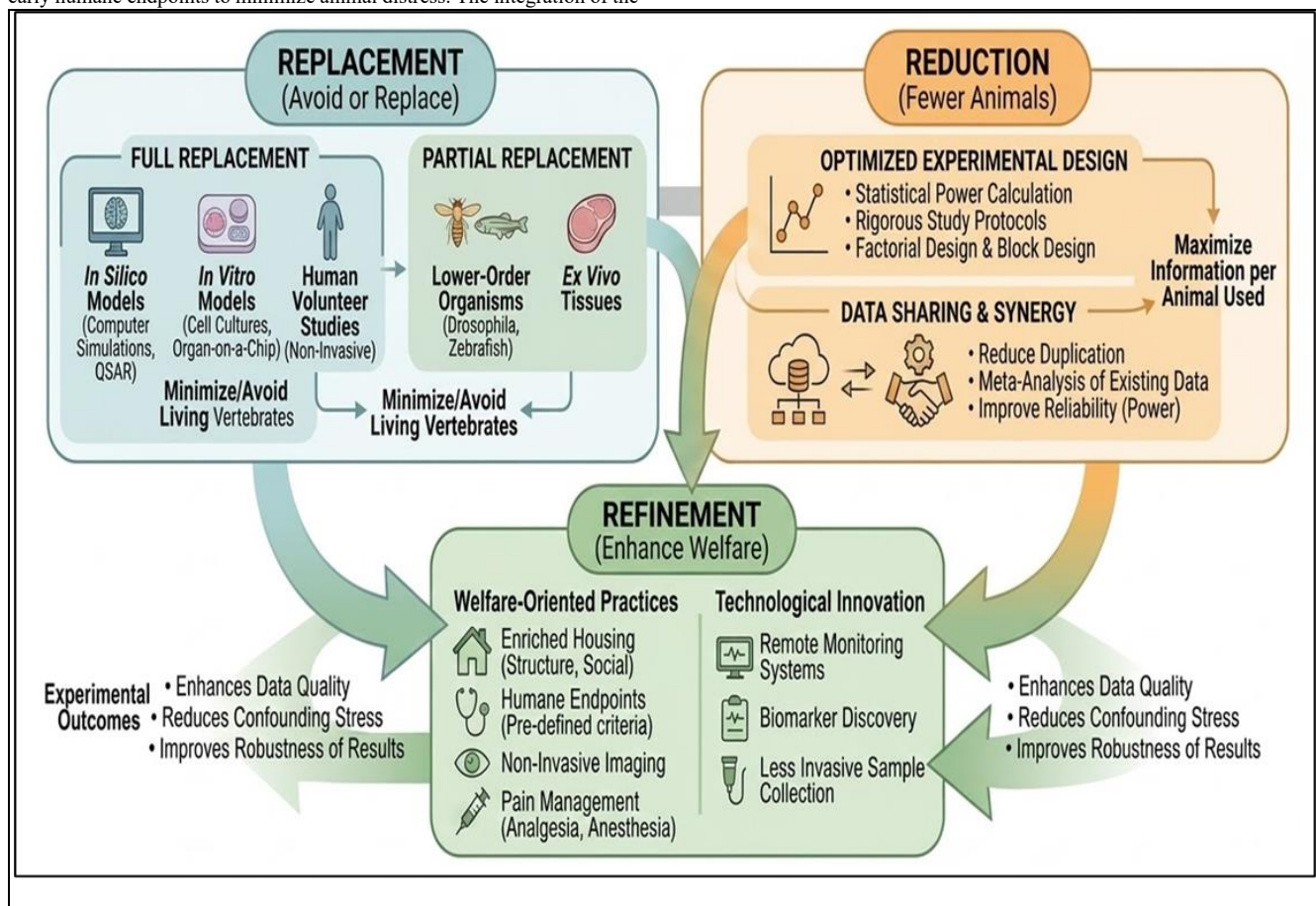


Fig. 1 - Conceptual framework illustrating the integration of the 3Rs (Replacement, Reduction, and Refinement) in biomedical research

Table 1 - Comparison of strategies implementing the 3rs

Principle	Key Strategies	Scientific Benefits	Ethical Impact	ReF.
Replacement	Organoids, organ-on-chip, AI models	Human-relevant data	Eliminates animal use	[20]
Reduction	Statistical design, imaging technologies	Improved data efficiency	Fewer animals required	[21]

Principle	Key Strategies	Scientific Benefits	Ethical Impact	ReF.
Refinement	Analgesia, enrichment, monitoring	Reduced variability	Improved welfare	[22]

Table 2 - Advantages and limitations of alternative models

Model	Advantages	Limitations	Ref.
3D cell cultures	Human-derived data	Limited systemic interaction	[23]
Organoids	Physiological relevance	High technical complexity	
Computational models	Rapid prediction	Dependent on data quality	

7. Future perspectives and research gaps

Despite significant scientific and regulatory progress, multiple challenges continue to impede the effective and widespread implementation of the 3Rs framework in biomedical research. At the technical level, limited standardization, validation constraints, and variability in the predictive performance of alternative experimental models restrict their broader acceptance. At the policy and regulatory level, established approval pathways and safety assessment requirements often favour traditional animal models, creating barriers to the adoption of innovative methodologies. In addition, disciplinary and institutional limitations, including insufficient interdisciplinary collaboration and gaps in specialized training in ethical experimental design, further influence implementation outcomes. Practical constraints such as infrastructure costs, technological accessibility, and data harmonization also contribute to uneven global adoption of humane research practices. Addressing these challenges requires the development of robust validation frameworks, harmonized regulatory guidelines, and strengthened collaborative research networks that integrate expertise from biomedical sciences, engineering, and computational disciplines. Strategic investment in capacity building and education in research ethics will be essential to facilitate the transition toward scientifically rigorous and ethically sustainable experimental paradigms.

8. Conclusion

The integration of the 3Rs framework represents a fundamental transformation in biomedical research ethics and methodology. Advances in biotechnology, computational sciences and engineering have created opportunities to replace traditional animal models with more predictive and human-relevant systems. Reduction strategies supported by improved experimental design and data sharing further enhance research efficiency, while refinement techniques ensure improved animal welfare. The multidisciplinary implementation of these principles not only aligns biomedical research with ethical responsibility but also contributes to greater scientific rigor and translational relevance. Continued innovation, policy support and international collaboration will be essential to accelerate the transition toward humane and scientifically robust research methodologies.

A.1. Abbreviations

3Rs:	Replacement, Reduction, Refinement
AI:	Artificial Intelligence
MRI:	Magnetic Resonance Imaging
PET:	Positron Emission Tomography
OECD:	Organisation for Economic Co-operation and Development

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