

Review Article

Artificial Intelligence in Experimental Surgery: Ethical Breakthroughs and Technological Innovations within In Silico Models

Amália Cinthia Meneses do Rêgo^{1,2}, Irami Araújo Filho^{1,2,3,4}

1. Institute of Teaching, Research, and Innovation, Liga Contra o Câncer, Brazil; 2. Postgraduate Program in Biotechnology, Potiguar University (UnP), Brazil; 3. Department of Surgery, Potiguar University (UnP), Brazil; 4. Department of Surgery, Universidade Federal do Rio Grande do Norte, Brazil

Integrating artificial intelligence (AI) into experimental surgery represents a transformative shift in biomedical research, offering innovative alternatives to traditional animal-based preclinical models. AI-driven methodologies, including computerized models and surgical simulations, enhance precision, reproducibility, and ethical compliance while reducing reliance on *in vivo* experimentation. This review systematically explores the role of AI in optimizing surgical procedures, operative techniques, and biomedical technology, analyzing its impact on surgical decision-making, predictive modeling, and training simulations. A comprehensive search was conducted across PubMed, Embase, Scopus, Web of Science, and SciELO, identifying studies on AI-enhanced surgical strategies, *in silico* models, and experimental validation techniques. The findings highlight AI's potential to replace animal testing, refine surgical training, and improve preclinical research accuracy. However, challenges remain, including data standardization, regulatory adaptation, and ethical considerations related to AI-driven surgical methodologies. Addressing these challenges requires interdisciplinary collaboration and the development of validated AI frameworks to support widespread implementation in experimental surgery. Future research should focus on standardizing AI applications, ensuring methodological transparency, and integrating AI models into clinical translation pathways. This review underscores AI's revolutionary role in shaping the future of surgical research, offering a path to more ethical, precise, and innovative experimental surgery.

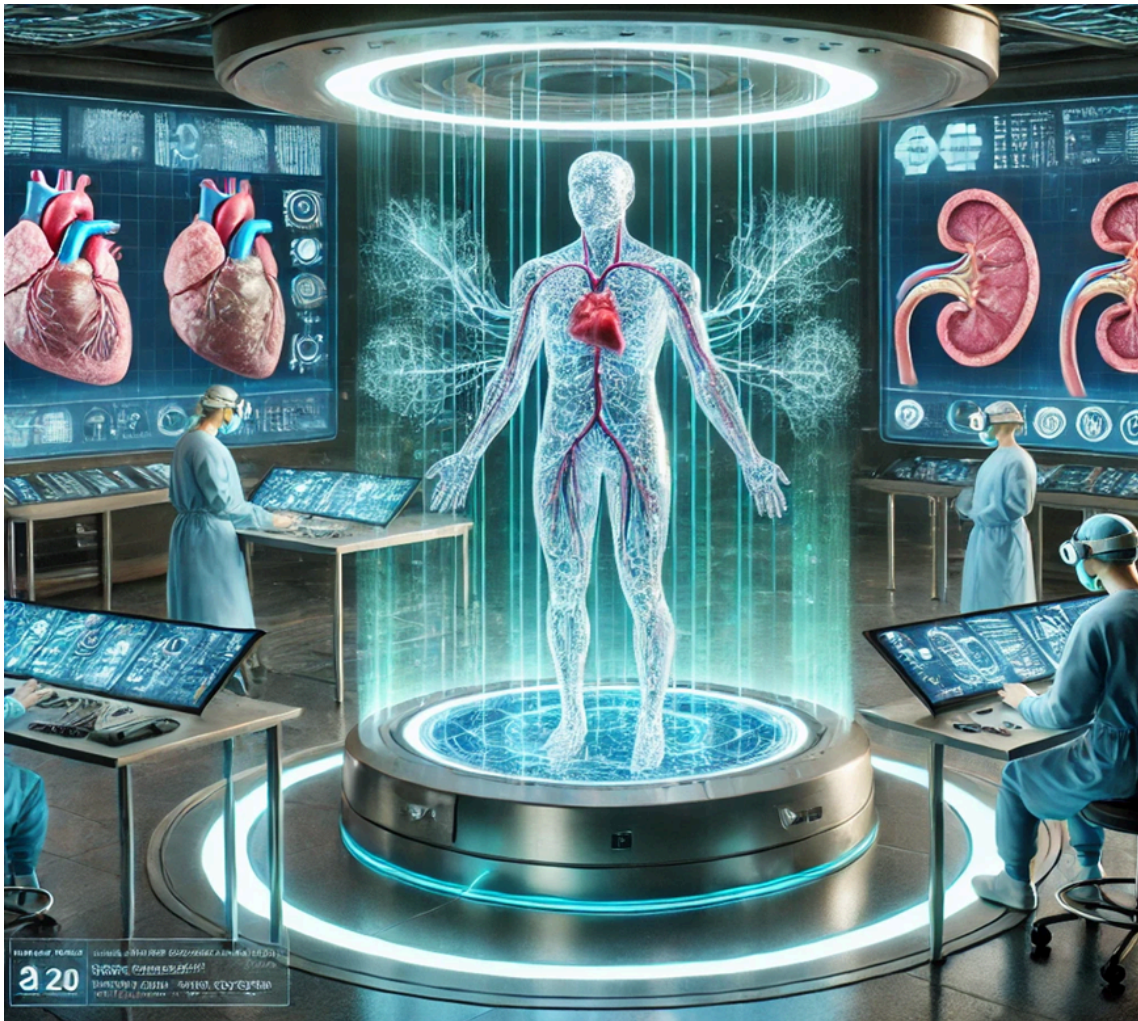


Figure 1. Futuristic AI-Assisted Surgical Research Lab

Source: <https://chatgpt.com/g/g-pmuQfob8d-image-generator>

Introduction

Artificial intelligence (AI) has become essential in advancing numerous scientific disciplines, significantly influencing fields that require data-driven decision-making, complex modeling, and predictive analytics^{[1][2][3]}. One of the most profound transformations is in experimental surgery, where AI is increasingly utilized to enhance precision, efficiency, and ethical responsibility^[4].

The historical reliance on animal models for surgical research has been foundational in advancing medical knowledge, yet it presents considerable ethical, scientific, and logistical challenges. Ethical concerns regarding the welfare of experimental animals, coupled with the translational gap between animal models and human applications, have driven the need for alternative methodologies^{[5][6][7][8]}.

Animal-based research's financial and temporal costs further complicate its viability as a sustainable approach. These limitations have led to an urgent search for more effective, ethical, and scientifically robust alternatives, with AI-driven in silico models emerging as a promising solution^{[9][10][11][12]}.

The evolution of AI in experimental surgery has been fueled by rapid advancements in computational power, machine learning algorithms, and the availability of extensive biomedical datasets. Human physiology can now be replicated with unprecedented accuracy in silico models, which simulate biological processes using AI^{[13][14][15][16]}.

These models leverage deep learning, neural networks, and advanced imaging techniques to construct highly detailed virtual representations of surgical scenarios. By enabling researchers to test hypotheses and refine surgical techniques without the need for live animal experimentation, AI-driven simulations provide a more ethical and scalable alternative to traditional methods^{[17][18][19][20]}. Furthermore, these technologies allow for real-time adjustments, dynamic modeling, and enhanced predictive analytics, ensuring that experimental results are more reliable and applicable to human clinical settings^{[21][22][23]}.

Ethical concerns surrounding animal testing have gained increasing global attention, leading to shifts in regulatory frameworks and scientific policies that encourage the development of alternative testing methods^{[24][25][26]}. Organizations advocating for ethical research and regulatory bodies have emphasized the need for compliance with the 3Rs principle—Reduction, Refinement, and Replacement, which seeks to minimize the use of animals in scientific studies^{[27][28][29][30]}. AI technologies align seamlessly with these principles by offering highly accurate predictive models that can either supplement or entirely replace traditional animal-based methodologies^{[31][32][33]}.

Notably, initiatives such as the ONTOX project and other AI-driven research programs in toxicology have demonstrated that computational approaches can provide superior predictive accuracy while adhering to ethical research standards. This has created momentum for adopting AI-based in silico approaches in toxicological studies, surgical research, and procedural simulations^{[34][35][36][37]}.

Beyond ethical considerations, the technological advantages of AI in experimental surgery extend to practical applications that improve research efficiency and reproducibility. Traditional experimental methodologies often suffer from inconsistencies due to biological variability in animal models, differences in experimental conditions, and human interpretation bias^{[38][39][40][41]}.

In contrast, AI-powered simulations provide highly standardized and reproducible conditions, ensuring that experimental variables remain controlled and precisely monitored. These models are particularly beneficial for complex surgical procedures that require high levels of precision, as AI can process vast datasets in real time, identify potential complications, and optimize surgical techniques with minimal error margins. By leveraging AI, researchers can conduct virtual surgeries to assess the impact of specific interventions, model post-operative healing trajectories, and predict patient-specific outcomes before clinical application^{[42][43][44][45]}.

Integrating multimodal data sources into computational models is one of the most significant breakthroughs in AI-driven surgical research. AI systems can process and analyze diverse datasets, including medical imaging (CT, MRI, ultrasound), genomic data, and patient-specific clinical parameters, to create highly detailed, personalized surgical simulations^{[46][47][48]}.

This integration enables a patient-centric approach to experimental surgery, where AI models can simulate procedures tailored to individual anatomical and physiological characteristics^{[49][50][51]}. This level of personalization enhances the translational applicability of research findings, bridging the gap between preclinical studies and real-world clinical interventions. AI-driven digital twin virtual replicas of human organs or full-body simulations—allow researchers to refine surgical techniques and test novel interventions with unparalleled precision^{[52][53][54][55]}.

Despite these advancements, implementing AI in experimental surgery is not without challenges. One major hurdle is the quality and availability of training data, as AI models require extensive datasets to learn and refine predictive capabilities. Inconsistencies in data collection, variations in imaging quality, and differences in annotation methodologies can affect the accuracy and reliability of AI-driven models^{[56][57][58][59]}.

The black-box nature of some deep learning algorithms presents interpretability issues, raising concerns about how AI reaches its conclusions. This is particularly critical in surgical research, where decision-making must be transparent and justifiable. Addressing these challenges requires the

development of explainable AI (XAI) models that offer transparent and interpretable outputs, enabling researchers and clinicians to trust and validate AI-generated predictions^{[60][61][62][63]}.

Adopting AI-driven in silico models in experimental surgery also necessitates adjustments in regulatory policies and validation protocols. Regulatory bodies, including the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), increasingly recognize AI-based methodologies as viable alternatives to traditional preclinical testing^{[64][65][66]}.

However, standardization of AI validation protocols is essential to ensure the widespread acceptance of these models in scientific and clinical research. Establishing guidelines for AI training, performance evaluation, and ethical considerations will be crucial in promoting the credibility and reliability of silico models in experimental surgery. Moreover, interdisciplinary collaboration between computer scientists, biomedical engineers, and surgeons is essential to refine AI applications and align them with existing research standards^{[67][68][69][70]}.

AI also revolutionizes surgical training and skill acquisition through immersive simulation platforms that enhance medical education. Integrated with AI-powered surgical models, virtual reality (VR) and augmented reality (AR) systems allow trainees to practice complex procedures in realistic environments. These platforms provide real-time feedback, assess surgical performance, and enable iterative learning without the ethical and practical limitations of using live subjects^{[71][72][73]}.

As AI technology continues to evolve, robotic-assisted surgery is another frontier where AI-driven algorithms are employed to enhance surgical precision, automate repetitive tasks, and optimize workflow efficiency in operating rooms. The synergy between AI, robotics, and silico modeling is poised to redefine the future of surgical innovation^{[74][75][76]}.

Despite AI's transformative potential, its integration into experimental surgery must be cautiously approached. Ethical concerns related to data privacy, algorithmic biases, and patient safety must be rigorously addressed to ensure responsible AI deployment. The future of AI in surgical research will depend on continuous innovation, regulatory adaptation, and multidisciplinary collaboration to refine methodologies and expand their applicability. While AI-driven in silico models offer a promising alternative to animal testing, ongoing research is needed to enhance their accuracy, interpretability, and clinical relevance^{[77][78][79]}.

The current state of AI in experimental surgery underscores a critical transition from traditional animal-based methodologies to ethically responsible, technology-driven research paradigms. AI has

the potential to accelerate surgical innovation, optimize training protocols, and improve patient outcomes, all while addressing ethical imperatives in biomedical research^{[80][81][82]}.

By advancing silico models, integrating multimodal data, and refining AI-driven predictive analytics, experimental surgery can move toward a future where precision, ethics, and innovation converge seamlessly. This review aims to comprehensively examine the transformative role of AI in experimental surgery, focusing on its implications for ethical research, technological advancements, and future directions in preclinical surgical modeling^{[83][84][85]}.

Through an in-depth exploration of current trends, challenges, and prospects, this study highlights AI's role in reshaping experimental surgery as a discipline that prioritizes scientific rigor and ethical responsibility (Figures 2-4).



Figure 2. The Future of Experimental Surgery with AI

Source: <https://chatgpt.com/g/g-pmuQfob8d-image-generator>

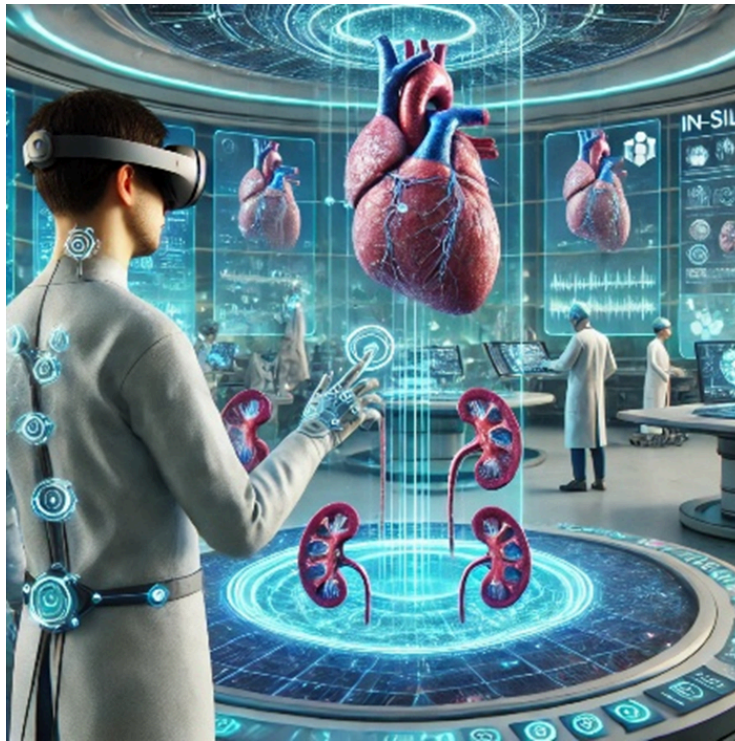


Figure 3. The Future of Experimental Surgery with AI

Source: <https://chatgpt.com/g/g-pmuQfob8d-image-generator>



Figure 4. The Future of Experimental Surgery with AI

Source: <https://chatgpt.com/g/g-pmuQfob8d-image-generator>

Methods

This review explored the role of artificial intelligence (AI) in experimental surgery, focusing on surgical procedures, operative techniques, computer simulation, computerized models, and biomedical technology as innovative alternatives to traditional animal-based experimental models. A systematic and comprehensive search was conducted across major scientific databases, including PubMed, Embase, Scopus, Web of Science, and SciELO, with additional sources identified through gray literature searches on Google Scholar. The search included studies published up to the present date to ensure a thorough assessment of the most recent and relevant evidence available.

The search strategy was designed using a combination of keywords and MeSH terms tailored to the primary focus areas of this review. The selected terms included "Artificial Intelligence," "Surgical Procedures, Operative," "Computer Simulation," "Computerized Models," and "Biomedical Technology." Boolean operators (AND, OR) were used to construct precise and efficient search strings, including a broad spectrum of relevant studies while maintaining specificity. Eligibility criteria were predefined to include various study designs, such as randomized controlled trials (RCTs), cohort

studies, case-control studies, cross-sectional studies, systematic reviews, and meta-analyses. Studies were selected based on whether they provided data on AI applications in optimizing surgical techniques, replacing traditional animal models, improving surgical decision-making, or advancing biomedical technology in experimental surgery. Articles were excluded if they did not directly investigate AI's role in experimental surgery, lacked methodological rigor, or presented only theoretical perspectives without empirical validation. No restrictions were applied regarding the type of surgical specialty or experimental setting, provided the study contained relevant information on AI-driven approaches in preclinical and experimental surgical research. The selection process was conducted by two independent reviewers who assessed the titles and abstracts of retrieved studies to identify potentially relevant articles. Discrepancies were resolved through discussion, and a third reviewer was consulted in cases of disagreement to achieve consensus. To minimize selection bias, reviewers remained blind to the authorship and institutional affiliations of the included studies. Full-text articles that met the inclusion criteria were retrieved and systematically evaluated for relevance to the review's objectives. Data extraction followed a standardized protocol to ensure consistency and reproducibility. Extracted data included study design, sample size, experimental model characteristics, AI methodologies applied, surgical techniques assessed, simulation models utilized, and AI's impact on experimental surgical outcomes. Thematic analysis categorized findings into key areas, including AI-enhanced surgical precision, the replacement of animal models, predictive modeling in surgical research, and AI-based medical simulation technologies. A critical evaluation of methodological quality was performed, emphasizing potential biases, limitations in study design, and inconsistencies in AI applications across different experimental surgical settings. Particular attention was given to identifying research gaps, including the lack of standardized protocols for AI integration in experimental surgery, variations in AI model validation, and limited comparative studies between AI-driven and conventional surgical techniques. Additionally, issues related to variability in machine learning algorithms, dataset availability, and potential biases in AI-based decision-making were systematically analyzed. The review also proposed future research directions, including the need for robust validation frameworks for AI applications in surgery, ethical considerations in AI-driven experimental research, the standardization of AI-based preclinical models, and interdisciplinary collaborations among surgeons, computational scientists, and regulatory agencies. By synthesizing current knowledge on AI applications in experimental surgery, this review highlights the transformative potential of AI-driven methodologies in replacing animal models, optimizing surgical techniques, and advancing biomedical technology. These insights guide future innovations in AI-

assisted surgical research, ensuring methodological rigor, ethical compliance, and translational relevance to clinical practice.

Results and Discussion

Medical Specialty	AI-Based Experimental Models
Neurosurgery	AI-driven brain simulations replicate tissue responses in neurosurgical procedures, replacing small animal models in traumatic brain injury research. Machine learning-based neurovascular models optimize microvascular anastomoses, reducing reliance on primates. AI-powered brain organoids simulate neuronal connections for preclinical testing of epilepsy and neurostimulation techniques.
Cardiovascular Surgery	Computational fluid dynamics (CFD) models simulate hemodynamic changes in valve replacement and coronary bypass procedures, eliminating the need for live animal circulation models. AI-driven virtual twin models predict postoperative complications in vascular surgeries. AI-powered heart-on-a-chip technology mimics myocardial contractility and response to pharmacological interventions, replacing canine and porcine heart models.
Gastrointestinal Surgery	AI-enhanced in silico gut models simulate peristalsis, acid secretion, and digestive processes for studying gastrointestinal surgical interventions. AI-powered tissue interaction models optimize suturing techniques for anastomosis, reducing reliance on porcine models. AI-driven biomechanical simulations of gastric sleeve and bariatric procedures predict tissue adaptation and metabolic responses.
Orthopedic Surgery	Deep learning-driven finite element analysis (FEA) models predict stress distribution in bones and joints, refining implant placement strategies. AI-based simulations of ligament and tendon injuries guide surgical repairs without requiring <i>in vivo</i> experimentation. AI-powered musculoskeletal simulations replace animal models in testing orthopedic prosthetic durability and spinal fusion procedures.
Plastic and Reconstructive Surgery	AI-based skin and soft tissue regeneration models assess wound healing and graft integration dynamics. AI-powered 3D bioprinted tissues provide realistic surgical practice environments, replacing live animal flap models in reconstructive surgery. AI-driven computational models predict scar formation and optimize laser-based scar revision techniques without requiring live animal testing.
Transplant Surgery	Machine learning-driven transplant rejection prediction models allow for immunosuppressive regimen optimization. AI-based kidney and liver perfusion simulations refine organ preservation protocols, replacing porcine and canine models.

Medical Specialty	AI-Based Experimental Models
	AI-powered organ decellularization and recellularization models predict successful graft integration, advancing xenotransplantation research without animal use.
Minimally Invasive and Robotic Surgery	AI-assisted robotic surgical training platforms replicate human tissue responses, allowing surgeons to refine techniques without using animal models. AI-driven autonomous robotic systems optimize laparoscopic and endoscopic procedures. AI-powered force-feedback mechanisms simulate tactile sensation for surgeons learning delicate robotic-assisted suturing techniques.
Oncologic Surgery	Deep learning cancer progression models predict tumor growth and resection margins, eliminating the need for xenograft models in surgical research. AI-powered organoid simulations refine experimental oncologic surgery approaches. AI-driven microfluidic tumor-on-a-chip models replicate metastasis and chemotherapeutic responses, replacing murine cancer models.
Ophthalmic Surgery	AI-based corneal and retinal simulation models replicate surgical manipulations in ophthalmic procedures. AI-driven predictive models of intraocular pressure dynamics reduce the need for animal testing in glaucoma surgery research. AI-powered retina organoid simulations replace live animal models for studying retinal detachment and macular degeneration interventions.
Regenerative Medicine and Tissue Engineering	AI-powered organ-on-a-chip models simulate microvascular perfusion and tissue regeneration, replacing large animal studies in regenerative medicine. AI-assisted bioprinter scaffolds offer an ethical alternative for studying tissue integration in experimental surgery. AI-driven stem cell differentiation models predict tissue formation and optimize scaffold seeding for complex tissue engineering applications.
Thoracic Surgery	AI-driven lung-on-a-chip models replace porcine and canine lung ventilation studies by mimicking alveolar gas exchange, surfactant dynamics, and fibrosis progression. AI-based virtual thoracic surgical simulations train surgeons in lung resection techniques without animal cadavers. AI-enhanced airflow modeling optimizes airway stent placement in bronchial disease treatment.
Urological Surgery	AI-powered urodynamic simulations predict bladder function, reducing reliance on animal voiding studies. AI-based prostate cancer progression models replace rodent prostatectomy experiments. AI-enhanced lithotripsy simulation platforms optimize shockwave and laser lithotripsy parameters for non-invasive urinary stone removal without live animal testing.

Medical Specialty	AI-Based Experimental Models
Hepatobiliary Surgery	AI-based hepatobiliary fluid dynamics models simulate bile duct obstructions, optimizing minimally invasive endoscopic drainage techniques. AI-driven hepatic tumor modeling platforms refine liver resection planning, eliminating the need for large animal liver studies. AI-powered liver-on-a-chip technology mimics hepatocellular carcinoma and drug metabolism studies, replacing primate liver models.
Endocrine Surgery	AI-enhanced thyroid and parathyroid surgical planning models replace rodent models in endocrine surgery. AI-based real-time nerve monitoring simulations optimize recurrent laryngeal nerve preservation techniques in thyroidectomy training. AI-powered adrenal tumor growth simulations refine pheochromocytoma surgical approaches without requiring animal validation.
Pediatric Surgery	AI-powered pediatric anatomical growth models simulate congenital malformation corrections, replacing fetal and neonatal animal models. AI-driven predictive models optimize pediatric organ transplant procedures without animal experiments. AI-enhanced neonatal surgical training platforms replicate infant tissue responses, refining surgical interventions for congenital disorders without reliance on animal neonates.

Table 1. AI-Based Experimental Surgical Models Replacing Animal Use

Source: Authors

Integrating artificial intelligence into experimental surgery represents a profound transformation in biomedical research, offering an alternative to traditional animal models while dramatically improving the precision, reproducibility, and ethical integrity of preclinical testing. For decades, animal models have been the cornerstone of experimental surgery, serving as essential tools for refining surgical techniques, studying pathophysiological mechanisms, and evaluating the efficacy of new therapeutic approaches (Table 1)^{[86][87][88]}.

However, these models come with intrinsic limitations, including interspecies biological discrepancies, substantial translational gaps, and ethical concerns regarding the welfare of laboratory animals. The development and implementation of AI-driven in silico models have emerged as a

groundbreaking alternative capable of overcoming these challenges while providing superior accuracy and predictive capability^{[20][21][22]}. By leveraging deep learning algorithms, computational modeling, and machine learning techniques, AI-driven approaches are revolutionizing surgical research, offering an unprecedented level of precision and adaptability that was previously unattainable^[89].

Artificial intelligence has evolved from a computational tool to an essential force in redefining experimental surgery. Modern AI systems can analyze vast datasets, integrating multimodal sources such as medical imaging, intraoperative sensor data, genomic profiles, and patient-specific physiological parameters to optimize surgical outcomes. Unlike conventional methods that rely on static data points, AI-based platforms operate as dynamic learning systems, continuously refining experimental methodologies based on real-time input^{[90][91][92]}.

AI-powered models facilitate the automation of complex decision-making processes, reducing human error and ensuring the standardization of experimental procedures. By utilizing predictive analytics, these systems allow for the real-time assessment of potential surgical complications, enabling preemptive adjustments to mitigate adverse outcomes before they manifest. This shift from empirical, animal-dependent experimentation to AI-enhanced simulations signifies a fundamental reconfiguration of surgical research, ushering in a new era of precision-driven, data-informed experimentation^{[93][94][95]}.

One of the most transformative applications of AI in experimental surgery is the development of high-fidelity in silico models. Unlike animal models, which often fail to accurately replicate human physiology, AI-driven simulations can generate precise virtual representations of human anatomy, tissue behavior, and pathological progression. These computational models can simulate intricate surgical procedures with remarkable accuracy, allowing researchers to test and refine techniques in a controlled, reproducible environment^{[96][97][98]}.

AI-powered in silico models have already demonstrated their efficacy in complex surgical fields, including liver resection, vascular anastomoses, robotic-assisted surgery, and oncologic interventions. By integrating computational fluid dynamics, biomechanical modeling, and AI-driven decision-support systems, these models allow for iterative refinements of surgical protocols before they are implemented in clinical practice^{[99][100]}.

The ability to conduct virtual surgical trials without exposing patients or laboratory animals to risk represents a paradigm shift in preclinical research, significantly enhancing experimental surgery's

ethical and scientific rigor. Despite the immense potential of AI in experimental surgery, its widespread adoption faces regulatory and ethical challenges that must be addressed to ensure its integration into mainstream research methodologies^{[100][101][102]}.

The current regulatory landscape remains centered mainly on traditional experimental models, creating obstacles to the validation and approval of AI-driven methodologies. Organizations such as the Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the World Health Organization (WHO) have yet to establish comprehensive guidelines for AI-based surgical simulations, leading to inconsistencies in acceptance across different research institutions^{[84][85][86][87]}.

Implementing AI-driven experimental models requires new regulatory frameworks that prioritize transparency in algorithmic decision-making, the standardization of machine learning training datasets, and the mitigation of predictive biases that could affect the reliability of results. Without explicit regulatory pathways, the transition from conventional animal-based preclinical research to AI-powered alternatives remains hindered, slowing the progression of this innovative approach^{[15][16][17][18]}.

The ethical implications of AI-based experimental models extend beyond eliminating animal testing. While AI significantly reduces ethical concerns associated with laboratory animal use, it introduces new challenges related to algorithmic bias, data privacy, and the interpretability of AI-generated findings. Biases in AI models can arise from disparities in training datasets, leading to inequitable predictions that disproportionately affect specific patient populations^{[26][27][28][29]}.

Additionally, AI-driven decision-making in surgical experimentation must be designed to ensure fairness, accountability, and transparency. Establishing ethical frameworks that regulate the use of AI in experimental surgery is critical for maintaining the integrity of preclinical research and ensuring that these models align with the highest standards of medical ethics^{[34][35][36][37]}.

An auspicious advancement in AI-driven experimental surgery is digital twin technology, which involves the creation of real-time, AI-enhanced virtual replicas of biological systems. Digital twins incorporate patient-specific data, continuously updated through real-time monitoring, enabling researchers and clinicians to simulate surgical interventions with unprecedented precision^{[41][42][43][44]}.

These models have been successfully applied in transplantation surgery to predict immune responses and assess graft viability, reducing the reliance on large-scale animal transplantation studies. In oncology, AI-powered digital twins are used to model tumor growth, determine optimal resection margins, and evaluate the impact of neoadjuvant therapies, demonstrating far greater predictive accuracy than traditional experimental models. The ability to conduct multiple patient-specific simulations before performing an actual surgical procedure represents a revolutionary advancement in precision medicine, transforming surgical research into a data-driven, patient-centered discipline^{[55][56][57][58]}.

The convergence of artificial intelligence and nanotechnology is another area poised to redefine experimental surgery. AI-driven nanorobotics has enabled the development of minimally invasive procedures at a molecular level, facilitating targeted drug delivery, nanoscale tissue repair, and the creation of bioengineered tissues that can replace damaged structures^{[60][61][62][63]}.

AI-powered nanoparticle modeling is being used to predict the biocompatibility of new materials, eliminating the need for extensive animal testing in biomaterial research. Furthermore, AI-enhanced nanosurgical simulations provide valuable insights into the interactions between biomaterials and living tissues, accelerating the development of next-generation surgical tools and implants. AI has also revolutionized experimental neurosurgery, particularly in developing brain-computer interfaces and neural network modeling^{[74][75][76][77]}.

AI-driven neural simulations have replaced primate-based neurophysiology studies, allowing for detailed cortical mapping, synaptic plasticity analysis, and neuroregeneration modeling. The use of AI in deep brain stimulation has enabled the creation of highly precise, individualized neuroprosthetics, significantly improving outcomes in conditions such as epilepsy, Parkinson's disease, and traumatic brain injuries^{[82][83][84][85]}. By harnessing AI-driven predictive modeling, researchers can optimize neurosurgical interventions with unprecedented accuracy, eliminating the variability and ethical concerns associated with traditional animal-based neurosurgical experimentation^[89].

The future of experimental surgery is inextricably linked to AI-driven innovations. As computational power advances, regulatory agencies refine their approaches to AI-based methodologies, and interdisciplinary collaborations strengthen, AI-driven surgical models will become the global standard for preclinical research^{[88][89][90]}.

The continued refinement of AI methodologies, the development of real-time adaptive deep learning algorithms, and the optimization of digital twin technology will solidify the transition from traditional experimental surgery to a fully AI-driven research paradigm. The ability of AI to integrate and analyze vast datasets, optimize surgical decision-making, and simulate complex biological processes with unprecedented accuracy is ushering in a new era of surgical experimentation that is fundamentally transforming the way research is conducted^{[94][95][96][97]}.

By embracing these technological advancements, the scientific community is moving toward a future in which experimental surgery is characterized by unparalleled precision, reproducibility, and ethical responsibility^[10]. The transition from animal-based models to AI-driven methodologies represents a scientific revolution and a moral imperative, ensuring that surgical research is conducted with the highest level of integrity while advancing medical knowledge in previously unimaginable ways^{[101][102][103]}.

The next decade will accelerate AI-driven experimental surgery as regulatory frameworks evolve, computational capabilities expand, and AI methodologies fully integrate into surgical research. The goal is to establish AI as the foundation of a new paradigm in experimental surgery, where data-driven decision-making, ethical responsibility, and scientific excellence converge to reshape the future of preclinical research^{[104][105]}.

Conclusion

Artificial intelligence has emerged as a transformative force in experimental surgery, fundamentally reshaping preclinical research methodologies and ethical frameworks. By leveraging computerized models, surgical simulations, and machine learning algorithms, AI enhances precision, reproducibility, and translational applicability, effectively addressing the limitations of traditional animal-based models. Implementing AI-driven approaches allows researchers to optimize surgical techniques, improve predictive modeling, and develop high-fidelity in silico simulations. This reduces the ethical and methodological concerns associated with in vivo experimentation.

Despite its significant advancements, integrating AI into experimental surgery presents challenges, including data standardization, regulatory validation, and algorithmic transparency. The absence of universally accepted guidelines for AI applications in preclinical research demands interdisciplinary collaboration among surgeons, computational scientists, and regulatory bodies to ensure

methodological rigor and ethical compliance. The development of standardized AI frameworks, real-time adaptive learning systems, and digital twin technology will further enhance AI's role in preclinical validation, surgical training, and clinical translation.

Future research should prioritize refining AI-driven experimental models, harmonizing regulatory standards, and ethically overseeing AI-generated surgical simulations. By embracing AI innovations, the scientific community will advance experimental surgery toward a more ethical, precise, and technologically sophisticated era, ultimately improving patient outcomes and translational research efficiency.

Statements and Declarations

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors thank the Federal University of Rio Grande do Norte, Potiguar University, and Liga Contra o Cancer for supporting this study.

References

1. [△]Sosnowski P, Sass P, Stanisławska-Sachadyn A, Krzemiński M, Sachadyn P. Between therapy effect and false-positive result in animal experimentation. *Biomed Pharmacother.* 2023 Apr; 160:114317. doi:10.1016/j.biopha.2023.114317.
2. [△]Hartung T. ToxAIcology – The evolving role of artificial intelligence in advancing toxicology and modernizing regulatory science. *ALTEX.* 2023; 40(4):559–570. doi:10.14573/altex.2309191.
3. [△]Drakos C, Manimangalam V, Burns C, Equils O. Artificial intelligence can help to make animal research redundant. *Nature.* 2024 Sep; 633(8029):286. doi:10.1038/d41586-024-02894-5.
4. [△]Alves VM, Auerbach SS, Kleinstreuer N, Rooney JP, Muratov EN, Rusyn I, Tropsha A, Schmitt C. Curated Data In – Trustworthy In Silico Models Out: The Impact of Data Quality on the Reliability of Artificial Intelligence Models as Alternatives to Animal Testing. *Altern Lab Anim.* 2021 May; 49(3):73–82. doi:10.1177/02611929211029635.

5. [△]Kim KB, Kwack SJ, Lee JY, Kacew S, Lee BM. Current opinion on risk assessment of cosmetics. *J Toxicol Environ Health B Crit Rev*. 2021 May 19; 24(4):137–161. doi:10.1080/10937404.2021.1907264.
6. [△]Liu Z, Huang R, Roberts R, Tong W. Toxicogenomics: A 2020 Vision. *Trends Pharmacol Sci*. 2019 Feb; 40(2):92–103. doi:10.1016/j.tips.2018.12.001.
7. [△]Vinken M, Benfenati E, Busquet F, Castell J, Clevert DA, de Kok TM, Dirven H, Fritsche E, Geris L, Gozalbes R, Hartung T, Jennen D, Jover R, Kandarova H, Kramer N, Krul C, Luechtefeld T, Masereeuw R, Roggen E, Schaller S, Vanhaecke T, Yang C, Piersma AH. Safer chemicals using less animals: kick-off of the European ONTOX project. *Toxicology*. 2021 Jun 30; 458:152846. doi:10.1016/j.tox.2021.152846.
8. [△]Sato K, Ikegaya Y. Challenges to Improve the Prediction Accuracy of the Non-clinical Tests for Human CNS Adverse Effects: Potentials of Artificial Intelligence and Human ESC/iPSC-derived Neurons. *Yakugaku Zasshi*. 2018; 138(6):807. Japanese. doi:10.1248/yakushi.17-00213-F.
9. [△]Bai X, Liu F, Liu Y, Li C, Wang S, Zhou H, Wang W, Zhu H, Winkler DA, Yan B. Toward a systematic exploration of nano-bio interactions. *Toxicol Appl Pharmacol*. 2017 May 15; 323:66–73. doi:10.1016/j.taap.2017.03.011.
10. ^{a, b}Diemar MG, Krul CAM, Teunis M, Busquet F, Kandarova H, Zajac JD, Vinken M, Roggen EL. Report of the First ONTOX Hackathon: Hack to Save Lives and Avoid Animal Suffering. The Use of Artificial Intelligence in Toxicology – A Potential Driver for Reducing/Replacing Laboratory Animals in the Future. *Altern Lab Anim*. 2025 Jan; 53(1):42–61. doi:10.1177/02611929241305112.
11. [△]Caloni F, De Angelis I, Hartung T. Replacement of animal testing by integrated approaches to testing and assessment (IATA): a call for in vivitrosi. *Arch Toxicol*. 2022 Jul; 96(7):1935–1950. doi:10.1007/s00204-022-03299-x.
12. [△]Hartung T, Maertens A, Luechtefeld T. E-validation – Unleashing AI for validation. *ALTEX*. 2024; 41(4):567–587. doi:10.14573/altex.2409211.
13. [△]Zhang W, Xia P, Liu S, Huang X, Zhao X, Liu Z, Dang H, Li X, Niu G. A coordinate positioning puncture method under robot-assisted CT-guidance: phantom and animal experiments. *Minim Invasive Ther Allied Technol*. 2022 Feb; 31(2):206–215. doi:10.1080/13645706.2020.1787451.
14. [△]Uesawa Y. AI-based QSAR Modeling for Prediction of Active Compounds in MIE/AOP. *Yakugaku Zasshi*. 2020; 140(4):499–505. Japanese. doi:10.1248/yakushi.19-00190-4.
15. ^{a, b}Funk C, Roth A. Current limitations and future opportunities for prediction of DILI from in vitro. *Arch Toxicol*. 2017 Jan; 91(1):131–142. doi:10.1007/s00204-016-1874-9.

16. ^{a, b}Maertens A, Antignac E, Benfenati E, Bloch D, Fritsche E, Hoffmann S, Jaworska J, Loizou G, McNally K, Piechota P, Roggen EL, Teunis M, Hartung T. The probable future of toxicology – probabilistic risk assessment. *ALTEX*. 2024; 41(2):273–281. doi:10.14573/altex.2310301.
17. ^{a, b}Hessel EVS, Staal YCM, Piersma AH. Design and validation of an ontology-driven animal-free testing strategy for developmental neurotoxicity testing. *Toxicol Appl Pharmacol*. 2018 Sep 1; 354:136–152. doi:10.1016/j.taap.2018.03.013.
18. ^{a, b}Ezendam J, Braakhuis HM, Vandebriel RJ. State of the art in non-animal approaches for skin sensitization testing: from individual test methods towards testing strategies. *Arch Toxicol*. 2016 Dec; 90(12):2861–2883. doi:10.1007/s00204-016-1842-4.
19. ^aIm JE, Lee JD, Kim HY, Kim HR, Seo DW, Kim KB. Prediction of skin sensitization using machine learning. *Toxicol In Vitro*. 2023 Dec; 93:105690. doi:10.1016/j.tiv.2023.105690.
20. ^{a, b}Knapen D, Vergauwen L, Villeneuve DL, Ankley GT. The potential of AOP networks for reproductive and developmental toxicity assay development. *Reprod Toxicol*. 2015 Aug 15; 56:52–5. doi:10.1016/j.reprotox.2015.04.003.
21. ^{a, b}Bell SM, Chang X, Wambaugh JE, Allen DG, Bartels M, Brouwer KLR, Casey WM, Choksi N, Ferguson S S, Fraczekiewicz G, Jarabek AM, Ke A, Lumen A, Lynn SG, Paini A, Price PS, Ring C, Simon TW, Sipes NS, Strickland CS, Strickland J, Troutman J, Wetmore BA, Kleinstreuer NC. In vitro to in vivo extrapolation for high throughput prioritization and decision making. *Toxicol In Vitro*. 2018 Mar; 47:213–227. doi:10.1016/j.tiv.2017.11.016.
22. ^{a, b}Maertens A, Luechtefeld T, Knight J, Hartung T. Alternative methods go green! Green toxicology as a sustainable approach for assessing chemical safety and designing safer chemicals. *ALTEX*. 2024; 41(1):3–19. doi:10.14573/altex.2312291.
23. ^aChung E, Wen X, Jia X, Ciallella HL, Aleksunes LM, Zhu H. Hybrid non-animal modeling: A mechanistic approach to predict chemical hepatotoxicity. *J Hazard Mater*. 2024 Jun 5; 471:134297. doi:10.1016/j.jhazmat.2024.134297.
24. ^aAmbe K, Ishihara K, Ochibe T, Ohya K, Tamura S, Inoue K, Yoshida M, Tohkin M. In Silico Prediction of Chemical-Induced Hepatocellular Hypertrophy Using Molecular Descriptors. *Toxicol Sci*. 2018 Apr 1; 162(2):667–675. doi:10.1093/toxsci/kfx287.
25. ^aZang Q, Paris M, Lehmann DM, Bell S, Kleinstreuer N, Allen D, Matheson J, Jacobs A, Casey W, Strickland J. Prediction of skin sensitization potency using machine learning approaches. *J Appl Toxicol*. 2017 Jul; 37(7):792–805. doi:10.1002/jat.3424.

26. ^{a, b}Wehr MM, Sarang SS, Rooseboom M, Boogaard PJ, Karwath A, Escher SE. *RespiraTox – Development of a QSAR model to predict human respiratory irritants*. Regul Toxicol Pharmacol. 2022 Feb; 128:105089. doi:10.1016/j.yrtph.2021.105089.
27. ^{a, b}Strickland J, Zang Q, Paris M, Lehmann DM, Allen D, Choksi N, Matheson J, Jacobs A, Casey W, Kleinstreuer N. *Multivariate models for prediction of human skin sensitization hazard*. J Appl Toxicol. 2017 Mar; 37(3):347–360. doi:10.1002/jat.3366.
28. ^{a, b}Hartung T, Kleinstreuer N. *Challenges and opportunities for validation of AI-based new approach methods*. ALTEX. 2025; 42(1):3–21. doi:10.14573/altex.2412291.
29. ^{a, b}Tung CW, Lin YH, Wang SS. *Transfer learning for predicting human skin sensitizers*. Arch Toxicol. 2019 Apr; 93(4):931–940. doi:10.1007/s00204-019-02420-x.
30. ^ΔWang Z, Fan M, Tao Q, Zhang Q, Lei S, Lv W. *Feasibility and safety study of advanced prostate biopsy robot system based on MR-TRUS Image flexible fusion technology in animal experiments*. SLAS Technol. 2024 Oct; 29(5):100184. doi:10.1016/j.slast.2024.100184.
31. ^ΔPatlewicz G, Fitzpatrick JM. *Current and Future Perspectives on the Development, Evaluation, and Application of in Silico Approaches for Predicting Toxicity*. Chem Res Toxicol. 2016 Apr 18; 29(4):438–51. doi:10.1021/acs.chemrestox.5b00388.
32. ^ΔNeff EP. *Animal behavior on auto*. Lab Anim (NY). 2019 Jun; 48(6):157–161. doi:10.1038/s41684-019-0312-z.
33. ^ΔChai W, Xie J, Zhang X, Yan T, Zhao Y, He C, Zhang Y. *[An animal experimental study on domestic robot-assisted total knee arthroplasty]*. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2020 Nov 15; 34(11):1376–1381. Chinese. doi:10.7507/1002-1892.202003173.
34. ^{a, b}Knight A. *Non-animal methodologies within biomedical research and toxicity testing*. ALTEX. 2008; 25(3):213–31. doi:10.14573/altex.2008.3.213.
35. ^{a, b}Enoch SJ, Cronin MT. *A review of the electrophilic reaction chemistry involved in covalent DNA binding*. Crit Rev Toxicol. 2010 Sep; 40(8):728–48. doi:10.3109/10408444.2010.494175.
36. ^{a, b}Srimathveeravalli G, Kim C, Petrisor D, Ezell P, Coleman J, Hricak H, Solomon SB, Stoianovici D. *MRI-safe robot for targeted transrectal prostate biopsy: animal experiments*. BJU Int. 2014 Jun; 113(6):977–85. doi:10.1111/bju.12335.
37. ^{a, b}Fitzpatrick JM, Roberts DW, Patlewicz G. *An evaluation of selected (Q) SARs/expert systems for predicting skin sensitisation potential*. SAR QSAR Environ Res. 2018 Jun; 29(6):439–468. doi:10.1080/1062936X.2018.1455223.

38. ^aJeon B, Lim MH, Choi TH, Kang BC, Kim S. A development of a graph-based ensemble machine learning model for skin sensitization hazard and potency assessment. *J Appl Toxicol.* 2022 Nov; 42(11):1832–1842. doi:10.1002/jat.4361.
39. ^aHan S, Ren M, Kuang T, Pang M, Guan D, Liu Y, Wang Y, Zhang W, Ye Z. Cerebellar Long Noncoding RNA Expression Profile in a Niemann–Pick C Disease Mouse Model. *Mol Neurobiol.* 2021 Nov; 58(11):5826–5836. doi:10.1007/s12035-021-02526-3.
40. ^aBai SZ, Ren N, Feng ZH, Xie R, Dong Y, Li ZW, Zhao YM. [Animal experiment on the accuracy of the Autonomous Dental Implant Robotic System]. *Zhonghua Kou Qiang Yi Xue Za Zhi.* 2021 Feb 9; 56(2):170–174. Chinese. doi:10.3760/cma.j.cn112144-20210107-00008.
41. ^{a, b}Verma RP, Matthews EJ. An in silico expert system for the identification of eye irritants. *SAR QSAR Environ Res.* 2015; 26(5):383–95. doi:10.1080/1062936X.2015.1039578.
42. ^{a, b}Altaner S, Jaeger S, Fotler R, Zemskov I, Wittmann V, Schreiber F, Dietrich DR. Machine learning prediction of cyanobacterial toxin (microcystin) toxicodynamics in humans. *ALTEX.* 2020; 37(1):24–36. doi:10.14573/altex.1904031.
43. ^{a, b}Neves M, Klippert A, Knöspel F, Rudeck J, Stolz A, Ban Z, Becker M, Diederich K, Grune B, Kahnau P, Ohnesorge N, Pucher J, Schönfelder G, Bert B, Butzke D. Automatic classification of experimental models in biomedical literature to support searching for alternative methods to animal experiments. *J Biomed Semantics.* 2023 Sep 1; 14(1):13. doi:10.1186/s13326-023-00292-w.
44. ^{a, b}Li H, Bai J, Zhong G, Lin H, He C, Dai R, Du H, Huang L. Improved defined approaches for predicting skin sensitization hazard and potency in humans. *ALTEX.* 2019; 36(3):363–372. doi:10.14573/altex.1809191.
45. ^aDi P, Zheng M, Yang T, Chen G, Ren J, Li X, Jiang H. Prediction of serious eye damage or eye irritation potential of compounds via consensus labeling models and active learning models based on uncertainty strategies. *Food Chem Toxicol.* 2022 Nov; 169:113420. doi:10.1016/j.fct.2022.113420.
46. ^aBasketter DA, Scholes EW, Chamberlain M, Barratt MD. An alternative strategy to the use of guinea pigs for the identification of skin sensitization hazard. *Food Chem Toxicol.* 1995 Dec; 33(12):1051–6. doi:10.1016/0278-6915(95)00073-9.
47. ^aWitte I, Plappert U, de Wall H, Hartmann A. Genetic toxicity assessment: employing the best science for human safety evaluation part III: the comet assay as an alternative to in vitro clastogenicity tests for early drug candidate selection. *Toxicol Sci.* 2007 May; 97(1):21–6. doi:10.1093/toxsci/kfl192.

48. [△]Patlewicz G, Kuseva C, Mehmed A, Popova Y, Dimitrova G, Ellis G, Hunziker R, Kern P, Low L, Ringeissen S, Roberts DW, Mekenyan O. TIMES-SS—recent refinements resulting from an industrial skin sensitization consortium. *SAR QSAR Environ Res.* 2014; 25(5):367–91. doi:10.1080/1062936X.2014.900520.
49. [△]Kang Y, Jeong B, Lim DH, Lee D, Lim KM. In silico prediction of the full United Nations Globally Harmonized System eye irritation categories of liquid chemicals by IATA-like bottom-up approach of random forest method. *J Toxicol Environ Health A.* 2021 Dec 2; 84(23):960–972. doi:10.1080/15287394.2021.1956661.
50. [△]Johansson H, Rydnert F, Kühnl J, Schepky A, Borrebaeck C, Lindstedt M. Genomic allergen rapid detection in-house validation—a proof of concept. *Toxicol Sci.* 2014 Jun; 139(2):362–70. doi:10.1093/toxsci/kfu046.
51. [△]Chedik L, Baybekov S, Marcou G, Cosnier F, Mourot-Bousquenaud M, Jacquenet S, Varnek A, Battais F. Benchmarking of BMDC assay and related QSAR study for identifying sensitizing chemicals. *Regul Toxicol Pharmacol.* 2024 May; 149:105623. doi:10.1016/j.yrtph.2024.105623.
52. [△]Shen Z, Zhong Y, Wang Y, Zhu H, Liu R, Yu S, Zhang H, Wang M, Yang T, Zhang M. A computational approach to estimate postmortem interval using postmortem computed tomography of multiple tissues based on animal experiments. *Int J Legal Med.* 2024 May; 138(3):1093–1107. doi:10.1007/s00414-023-03127-6.
53. [△]Verma RP, Matthews EJ. Estimation of the chemical-induced eye injury using a weight-of-evidence (WoE) battery of 21 artificial neural network (ANN) c-QSAR models (QSAR-21): part I: irritation potential. *Regul Toxicol Pharmacol.* 2015 Mar; 71(2):318–30. doi:10.1016/j.yrtph.2014.11.011.
54. [△]Hostýnek JJ, Magee PS, Maibach HI. QSAR predictive of contact allergy: scope and limitations. *Curr Probl Dermatol.* 1996; 25:18–27. doi:10.1159/000425511.
55. [△]^aPrieto P, Kinsner-Ovaskainen A, Stanzel S, Albella B, Artursson P, Campillo N, Cecchelli R, Cerrato L, Díaz L, Di Consiglio E, Guerra A, Gombau L, Herrera G, Honegger P, Landry C, O'Connor JE, Páez JA, Quintas G, Svensson R, Turco L, Zurich MG, Zurbano MJ, Kopp-Schneider A. The value of selected in vitro and in silico methods to predict acute oral toxicity in a regulatory context: results from the European Project ACuteTox. *Toxicol In Vitro.* 2013 Jun; 27(4):1357–76. doi:10.1016/j.tiv.2012.07.013.
56. [△]^bYiannakou M, Menikou G, Yiallouras C, Ioannides C, Damianou C. MRI guided focused ultrasound robotic system for animal experiments. *Int J Med Robot.* 2017 Dec; 13(4). doi:10.1002/rcs.1804.
57. [△]^a^bMacmillan DS, Chilton ML. A defined approach for predicting skin sensitisation hazard and potency based on the guided integration of in silico, in chemico and in vitro data using exclusion criteria. *Regul*

Toxicol Pharmacol. 2019 Feb; 101:35–47. doi:10.1016/j.yrtph.2018.11.001.

58. ^aDi P, Yin Y, Jiang C, Cai Y, Li W, Tang Y, Liu G. Prediction of the skin sensitizing potential and potency of compounds via mechanism-based binary and ternary classification models. *Toxicol In Vitro.* 2019 Sep; 59:204–214. doi:10.1016/j.tiv.2019.01.004.
59. ^ΔMacmillan DS, Canipa SJ, Chilton ML, Williams RV, Barber CG. Predicting skin sensitization using a decision tree integrated testing strategy with an in silico model and in chemico/in vitro assays. *Regul Toxicol Pharmacol.* 2016 Apr; 76:30–8. doi:10.1016/j.yrtph.2016.01.009.
60. ^aLuechtefeld T, Marsh D, Rowlands C, Hartung T. Machine Learning of Toxicological Big Data Enables Read-Across Structure Activity Relationships (RASAR) Outperforming Animal Test Reproducibility. *Toxicol Sci.* 2018 Sep; 165(1):198–212. doi:10.1093/toxsci/kfy152.
61. ^aVerma RP, Matthews EJ. Estimation of the chemical-induced eye injury using a Weight-of-Evidence (WoE) battery of 21 artificial neural network (ANN) c-QSAR models (QSAR-21): part II: corrosion potential. *Regul Toxicol Pharmacol.* 2015 Mar; 71(2):331–6. doi:10.1016/j.yrtph.2014.12.004.
62. ^aLuo X, Xu T, Ngan DK, Xia M, Zhao J, Sakamuru S, Simeonov A, Huang R. Prediction of chemical-induced acute toxicity using in vitro assay data and chemical structure. *Toxicol Appl Pharmacol.* 2024 Nov; 492:117098. doi:10.1016/j.taap.2024.117098.
63. ^aAmbe K, Suzuki M, Ashikaga T, Tohkin M. Development of quantitative model of a local lymph node assay for evaluating skin sensitization potency applying machine learning CatBoost. *Regul Toxicol Pharmacol.* 2021 Oct; 125:105019. doi:10.1016/j.yrtph.2021.105019.
64. ^ΔDesprez B, Birk B, Blaauboer B, Boobis A, Carmichael P, Cronin MTD, Curie R, Daston G, Hubesch B, Jennings P, Klaric M, Kroese D, Mahony C, Ouédraogo G, Piersma A, Richarz AN, Schwarz M, van Benthem J, van de Water B, Vinken M. A mode-of-action ontology model for safety evaluation of chemicals: Outcome of a series of workshops on repeated dose toxicity. *Toxicol In Vitro.* 2019 Sep; 59:44–50. doi:10.1016/j.tiv.2019.04.005.
65. ^ΔYang CZ, Casey W, Stoner MA, Kollessery GJ, Wong AW, Bittner GD. A robotic MCF-7:WS8 cell proliferation assay to detect agonist and antagonist estrogenic activity. *Toxicol Sci.* 2014 Feb; 137(2):335–49. doi:10.1093/toxsci/kft250.
66. ^ΔLuechtefeld T, Maertens A, McKim JM, Hartung T, Kleensang A, Sá-Rocha V. Probabilistic hazard assessment for skin sensitization potency by dose-response modeling using feature elimination instead of quantitative structure-activity relationships. *J Appl Toxicol.* 2015 Nov; 35(11):1361–1371. doi:10.1002/jat.3172.

67. [△]Golla S, Madihally S, Robinson RL Jr, Gasem KA. Quantitative structure–property relationships modeling of skin irritation. *Toxicol In Vitro*. 2009 Feb;23(1):176–84. doi:10.1016/j.tiv.2008.10.013.
68. [△]Ulrich R, Kalkuhl A, Deschl U, Baumgärtner W. Machine learning approach identifies new pathways associated with demyelination in a viral model of multiple sclerosis. *J Cell Mol Med*. 2010 Jan;14(1–2):434–48. doi:10.1111/j.1582–4934.2008.00646.x.
69. [△]Lehmann U, Stark H, Bartels S, Schlue J, Büsche G, Kreipe H. Genome–wide DNA methylation profiling is able to identify prefibrotic PMF cases at risk for progression to myelofibrosis. *Clin Epigenetics*. 2021 Feb 4;13(1):28. doi:10.1186/s13148–021–01010–y.
70. [△]Trifunović J, Borčić V, Vukmirović S, Vasović V, Mikov M. Bile acids and their oxo derivatives: environmentally safe materials for drug design and delivery. *Drug Chem Toxicol*. 2017 Oct;40(4):397–405. doi:10.1080/01480545.2016.1244680.
71. [△]Johansson H, Lindstedt M, Albrekt AS, Borrebaeck CA. A genomic biomarker signature can predict skin sensitizers using a cell–based in vitro alternative to animal tests. *BMC Genomics*. 2011 Aug 8;12:399. doi:10.1186/1471–2164–12–399.
72. [△]Forreryd A, Zeller KS, Lindberg T, Johansson H, Lindstedt M. From genome–wide arrays to tailor–made biomarker readout—Progress towards routine analysis of skin sensitizing chemicals with GARD. *Toxicol In Vitro*. 2016 Dec;37:178–188. doi:10.1016/j.tiv.2016.09.013.
73. [△]Langton K, Patlewicz GY, Long A, Marchant CA, Basketter DA. Structure–activity relationships for skin sensitization: recent improvements to Derek for Windows. *Contact Dermatitis*. 2006 Dec;55(6):342–7. doi:10.1111/j.1600–0536.2006.00969.x.
74. [△][‡]Forreryd A, Norinder U, Lindberg T, Lindstedt M. Predicting skin sensitizers with confidence—Using conformal prediction to determine applicability domain of GARD. *Toxicol In Vitro*. 2018 Apr;48:179–187. doi:10.1016/j.tiv.2018.01.021.
75. [△][‡]Hartung T, Bremer S, Casati S, Coecke S, Corvi R, Fortaner S, Gribaldo L, Halder M, Hoffmann S, Roi AJ, Prieto P, Sabbioni E, Scott L, Worth A, Zuang V. A modular approach to the ECVAM principles on test validity. *Altern Lab Anim*. 2004 Nov;32(5):467–72. doi:10.1177/026119290403200503.
76. [△][‡]Keiper A. The age of neuroelectronics. *New Atlantis*. 2006 Winter; 11:4–41.
77. [△][‡]Rosenkranz HS, Klopman G. CASE, the computer–automated structure evaluation system, as an alternative to extensive animal testing. *Toxicol Ind Health*. 1988 Dec;4(4):533–40. doi:10.1177/074823378800400411.

78. ^aRidings JE, Barratt MD, Cary R, Earnshaw CG, Eggington CE, Ellis MK, Judson PN, Langowski JJ, Marchant CA, Payne MP, Watson WP, Yih TD. Computer prediction of possible toxic action from chemical structure: an update on the DEREK system. *Toxicology*. 1996 Jan 8;106(1-3):267-79. doi:10.1016/0300-483x(95)03190-q.
79. ^aHirota M, Fukui S, Okamoto K, Kurotani S, Imai N, Fujishiro M, Kyotani D, Kato Y, Kasahara T, Fujita M, Toyoda A, Sekiya D, Watanabe S, Seto H, Takenouchi O, Ashikaga T, Miyazawa M. Evaluation of combinations of in vitro sensitization test descriptors for the artificial neural network-based risk assessment model of skin sensitization. *J Appl Toxicol*. 2015 Nov;35(11):1333-47. doi:10.1002/jat.3105.
80. ^aWorth AP. ECVAM's activities on computer modeling and integrated testing. *Altern Lab Anim*. 2002 Dec;30 Suppl 2:133-7. doi:10.1177/026119290203002S22.
81. ^aEllison CM, Enoch SJ, Cronin MT, Madden JC, Judson P. Definition of the applicability domains of knowledge-based predictive toxicology expert systems by using a structural fragment-based approach. *Altern Lab Anim*. 2009 Nov;37(5):533-45. doi:10.1177/026119290903700510.
82. ^{a, b}Hartung T, Hoffmann S. Food for thought... on in silico methods in toxicology. *ALTEX*. 2009;26(3):155-66. doi:10.14573/altex.2009.3.155.
83. ^{a, b}Ristolainen A, Colucci G, Kruusmaa M. A phantom pig abdomen as an alternative for testing robotic surgical systems: our experience. *Altern Lab Anim*. 2013 Nov;41(5):359-67. doi:10.1177/026119291304100506.
84. ^{a, b, c}Gerner I, Zinke S, Graetschel G, Schlede E. Development of a decision support system for the introduction of alternative methods into local irritancy/corrosivity testing strategies. Creation of fundamental rules for a decision support system. *Altern Lab Anim*. 2000 Sep-Oct;28(5):665-98. doi:10.1177/026119290002800505.
85. ^{a, b, c}Ringeissen S, Marrot L, Note R, Labarussiat A, Imbert S, Todorov M, Mekenyan O, Meunier JR. Development of a mechanistic SAR model for the detection of phototoxic chemicals and use in an integrated testing strategy. *Toxicol In Vitro*. 2011 Feb;25(1):324-34. doi:10.1016/j.tiv.2010.09.017.
86. ^{a, b}Lunghini F, Marcou G, Azam P, Horvath D, Patoux R, Van Miert E, Varnek A. Consensus models to predict oral rat acute toxicity and validation on a dataset coming from the industrial context. *SAR QSAR Environ Res*. 2019 Dec;30(12):879-897. doi:10.1080/1062936X.2019.1672089.
87. ^{a, b}Calleja MC, Geladi P, Persoone G. QSAR models for predicting the acute toxicity of selected organic chemicals with diverse structures to aquatic non-vertebrates and humans. *SAR QSAR Environ Res*. 1994;2(3):193-234. doi:10.1080/10629369408029903.

88. ^{a, b}Fitzpatrick JM, Patlewicz G. Application of IATA—A case study in evaluating the global and local performance of a Bayesian network model for skin sensitization. *SAR QSAR Environ Res.* 2017 Apr;28(4):297–310. doi:10.1080/1062936X.2017.1311941.
89. ^{a, b, c}Liu Z, Huang R, Roberts R, Tong W. Toxicogenomics: A 2020 Vision. *Trends Pharmacol Sci.* 2019 Feb;40(2):92–103. doi:10.1016/j.tips.2018.12.001.
90. ^{a, b}Bai X, Liu F, Liu Y, Li C, Wang S, Zhou H, Wang W, Zhu H, Winkler DA, Yan B. Toward a systematic exploration of nano–bio interactions. *Toxicol Appl Pharmacol.* 2017 May 15;323:66–73. doi:10.1016/j.taap.2017.03.011.
91. ^ΔAlves VM, Auerbach SS, Kleinstreuer N, Rooney JP, Muratov EN, Rusyn I, Tropsha A, Schmitt C. Curated Data In – Trustworthy In Silico Models Out: The Impact of Data Quality on the Reliability of Artificial Intelligence Models as Alternatives to Animal Testing. *Altern Lab Anim.* 2021 May; 49(3):73–82. doi:10.1177/02611929211029635.
92. ^ΔKim KB, Kwack SJ, Lee JY, Kacew S, Lee BM. Current opinion on risk assessment of cosmetics. *J Toxicol Environ Health B Crit Rev.* 2021 May 19; 24(4):137–161. doi:10.1080/10937404.2021.1907264.
93. ^ΔDrakos C, Manimangalam V, Burns C, Equils O. Artificial intelligence can help to make animal research redundant. *Nature.* 2024 Sep; 633(8029):286. doi:10.1038/d41586-024-02894-5.
94. ^{a, b}Vinken M, Benfenati E, Busquet F, Castell J, Clevert DA, de Kok TM, Dirven H, Fritsche E, Geris L, Gozalbes R, Hartung T, Jennen D, Jover R, Kandarova H, Kramer N, Krul C, Luechtefeld T, Masereeuw R, Roggen E, Schaller S, Vanhaecke T, Yang C, Piersma AH. Safer chemicals using less animals: kick-off of the European ONTOX project. *Toxicology.* 2021 Jun 30; 458:152846. doi:10.1016/j.tox.2021.152846.
95. ^{a, b}Sato K, Ikegaya Y. Challenges to Improve the Prediction Accuracy of the Non-clinical Tests for Human CNS Adverse Effects: Potentials of Artificial Intelligence and Human ESC/iPSC-derived Neurons. *Yakugaku Zasshi.* 2018; 138(6):807. doi:10.1248/yakushi.17-00213-F.
96. ^{a, b}Diemar MG, Krul CAM, Teunis M, Busquet F, Kandarova H, Zajac JD, Vinken M, Roggen EL. Report of the First ONTOX Hackathon: Hack to Save Lives and Avoid Animal Suffering. The Use of Artificial Intelligence in Toxicology – A Potential Driver for Reducing/Replacing Laboratory Animals in the Future. *Altern Lab Anim.* 2025 Jan; 53(1):42–61. doi:10.1177/02611929241305112.
97. ^{a, b}Hartung T. ToxAIcology – The evolving role of artificial intelligence in advancing toxicology and modernizing regulatory science. *ALTEx.* 2023; 40(4):559–570. doi:10.14573/altex.2309191.
98. ^ΔCaloni F, De Angelis I, Hartung T. Replacement of animal testing by integrated approaches to testing and assessment (IATA): a call for in vivitrosi. *Arch Toxicol.* 2022 Jul; 96(7):1935–1950. doi:10.1007/s0020

4-022-03299-x.

99. ^ΔKnapen D, Vergauwen L, Villeneuve DL, Ankley GT. The potential of AOP networks for reproductive and developmental toxicity assay development. *Reprod Toxicol*. 2015 Aug 15; 56:52-5. doi:10.1016/j.reprotox.2015.04.003.
100. ^{a, b}Im JE, Lee JD, Kim HY, Kim HR, Seo DW, Kim KB. Prediction of skin sensitization using machine learning. *Toxicol In Vitro*. 2023 Dec; 93:105690. doi:10.1016/j.tiv.2023.105690.
101. ^{a, b}Hessel EVS, Staal YCM, Piersma AH. Design and validation of an ontology-driven animal-free testing strategy for developmental neurotoxicity testing. *Toxicol Appl Pharmacol*. 2018 Sep 1; 354:136-152. doi:10.1016/j.taap.2018.03.013.
102. ^{a, b}Hartung T, Maertens A, Luechtefeld T. E-validation – Unleashing AI for validation. *ALTEX*. 2024; 41(4):567-587. doi:10.14573/altex.2409211.
103. ^ΔTung CW, Lin YH, Wang SS. Transfer learning for predicting human skin sensitizers. *Arch Toxicol*. 2019 Apr; 93(4):931-940. doi:10.1007/s00204-019-02420-x.
104. ^ΔChung E, Wen X, Jia X, Ciallella HL, Aleksunes LM, Zhu H. Hybrid non-animal modeling: A mechanistic approach to predict chemical hepatotoxicity. *J Hazard Mater*. 2024 Jun 5; 471:134297. doi:10.1016/j.jhazmat.2024.134297.
105. ^ΔEzendam J, Braakhuis HM, Vandebriel RJ. State of the art in non-animal approaches for skin sensitization testing: from individual test methods towards testing strategies. *Arch Toxicol*. 2016 Dec; 90(12):2861-2883. doi:10.1007/s00204-016-1842-4.

Declarations

Funding: The authors thank the Federal University of Rio Grande do Norte, Potiguar University, and Liga Contra o Cancer for supporting this study.

Potential competing interests: No potential competing interests to declare.