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Role of Modern Technologies in Reshaping New Age Toxicology

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Abstract

Toxicology defines the adverse effects of chemicals, substances, or environmental agents on living organisms. Traditional toxicology has undergone dramatic evolution from basic observational toxicology to new age toxicology, that relies more on mechanisms at cellular and molecular levels. Due to significant increase in the number of new chemicals (drugs, cosmetics, healthcare and consumer products, and environmental chemicals), toxicity testing has become very expensive, and time and labor-intensive process that consumes a large number of animals. This prompted evolving new alternative approaches that could significantly improve the process of toxicity testing. Integration of high-end automated technologies like High-throughput Screening, Omics Technologies (Genomics, Transcriptomics, Proteomics, and Metabolomics), Organ-on-a Chip and Microfluidics Models, 3-D (three dimensional) Bioprinting, Advanced Imaging Systems, Computational Modeling, and other technologies have revolutionized the new age toxicology. This remarkably enhanced human productivity by improving the speed, sensitivity, specificity, and reproducibility of toxicity testing in compliance with regulatory guidelines. This article provides an overview of various modern technologies attributable to reshaping of new age toxicology.

Keywords: Traditional toxicology; New age toxicology; Toxicity testing; Alternative methods; Modern technologies

1. Introduction

Toxicology defines the adverse effects of chemical, physical, or biological agents on living organisms. Toxicology has undergone dramatic evolution from basic observational toxicology to new age toxicology, that addresses mechanisms underlying the toxic manifestations at cellular and molecular levels [1]. In compliance with the regulatory guidelines, all new drug molecules, cosmetics, healthcare and consumer products, and environmental chemicals have to undergo mandatory toxicity testing for safety assessment [2,3]. Traditional toxicity testing using large number of animals was expensive, and time and labor-intensive process that often failed to detect toxicants or its metabolites in biological samples, and also suffered from many ethical issues [3-6]. This prompted evolving new alternative approaches that could improve the speed, sensitivity, specificity, and reproducibility of toxicity testing conforming to regulatory guidelines. Therefore, a paradigm shift from traditional to new age toxicology was realized. This resulted in a revolution in toxicity testing process that enhanced human productivity. Advent of high-end automated technologies like High-throughput Screening (HTS), Omics Technologies (Genomics, Transcriptomics, Proteomics, and Metabolomics), Organ-on-a Chip and Microfluidics Models, 3-D (three dimensional) Bioprinting, Advanced Imaging Systems, Computational Modeling, and other technologies revolutionized the new age toxicology. This hastened the process of generating complex and voluminous toxicity data, and their interpretation thereof [5-7]. This article provides an overview of various modern technologies that are responsible for reshaping the new age toxicology.

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2. New age toxicology

The new age toxicology relates to scientific advancements for more rigorous safety evaluation and risk assessment of human-relevant toxicants. Toxicity testing previously relied more on lower-order vertebrates for their anatomical, physiological, and behavioral analogy with humans. Such in vivo (whole animal) studies were considered 'Gold Standards' as they provided key information on the fate of chemicals in living organisms [4-7]. However, owing to several concerns associated with animal welfare and ethics, rule of '3 Rs' (reduction, replacement, and refinement) was advocated to minimize the use of animals in toxicity assays [6-8]. The recommendations included reduction in the number of animal experiments to minimum necessary, refinement in experiments to cause less sufferings, and replace in vivo experiments with simpler test methods. Alternatively, in vitro tests utilizing isolated primary cells, specialized cell lines, and tissue cultures were employed in experimental toxicology [9-11]. However, such tests were also plagued by several constraints [12], and many researchers started complimenting the in vitro findings with in vivo data [13]. Toxicology was not influenced by the revolution in computer science until in silico testing of chemicals was introduced [14-18]. In silico analysis refers to conducting scientific experiments with the aid of computer modeling or simulation software. Discovery of new drugs largely relies on in silico tools, that report the structure-activity relationship (SARs), molecular docking, and absorption, distribution, metabolism, elimination and toxicity (ADME/T) analysis of human relevance. Such predictive tools are mainly used for ligand-based drug design, structure-based drug design, and chromogenic assays [19-22]. Under the ToxCast programme of the United States Environmental Protection Agency (USEPA), about 1065 chemicals and drug substances were tested using in silico modelling and human pluripotent stem cells (hPSCs) assay to predict in vivo developmental toxicity with ~80% accuracy and >84% specificity [23]. Further refinement in analytical technologies involving automated processes was warranted to increase efficiency, accuracy, and productivity. With the integration of computer software, cellular growth, development of human nervous and motor control systems, and process of disease transmission and disease progression could be predicted by simulation techniques [24]. Automation also resulted in accurate analysis and interpretation of voluminous toxicity data. Further, advent of several state-of-the-art technologies immensely influenced the new age toxicology, that experienced improved human capabilities and productivity [24-26].

3. Modern technologies

Mankind is usually exposed to thousands of chemicals and millions of mixtures during life time. This poses a great challenge for the toxicologists to evolve new technologies to efficiently and rapidly evaluate such vast number of chemicals for their toxicity and safety. Traditional technologies employed for rapid analyses of samples included spectrophotometry, chromatography, immunology, gas chromatography-mass spectrometry, and metal analysis techniques. Some of the supportive tools include automated albumin, hematology, blood biochemistry, blood gas, metabolism, and amino acid analyzers [1-3,14,15]. Revolution in computer applications in the recent past resulted in emergence of high-end technologies that dramatically influenced toxicological research, particularly in toxicogenomic, epigenomics, bioinformatics, and computational biology. These new technologies remarkably enhanced the speed, accuracy, and efficiency of toxicity assessment [22,24-26]. Emergence of such technologies has transformed the traditional in vivo toxicity testing to more reliable in vitro techniques, and data obtained there off are more translatable to humans [19-22]. Some of the modern technologies discussed below have several applications in toxicology research, including risk assessment, biomarker discovery, and developing personalized medicines, etc.[22,24-28].

3.1. High-throughput Screening (HTS)

HTS is a powerful fully automated system that uses robotics, liquid handlers, and detectors (multi-well plate readers) to rapidly test the chemicals for potential toxicity against specific biological targets (enzymes or receptors). HTS has significantly improved the accuracy and efficiency of screening of large number of chemicals for risk assessment [27]. It is crucial for screening environmental toxins, drug discovery, biomarker research, and genomics to find lead molecules. However, HTS is not free from certain drawbacks like low specificity (false positives/negatives) and inability to address the complex interactions in vivo. Also, HTS consumes large amount of chemicals that cannot be related to real-exposure scenario [28]. Nevertheless, equipped with sensitive detectors (absorbance, fluorescence, luminescence) and sophisticated software, HTS remains a preferred tool for screening large number of chemicals in pharmaceutical, cosmetic, consumer product, and many other industries [27-29].

3.2. Omics Technologies

Omics technologies are high-throughput scientific tools that provide a comprehensive and holistic view of biological systems by studying large sets of molecules like DNA (genomics), RNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). When applied to toxicology, omics is referred as toxicogenomics, where it can identify

biomarkers of toxicity at molecular levels to delineate mechanisms of toxicity and individual susceptibility [30,31]. Omics technologies play a crucial role in advances in personalized medicine, drug discovery, disease diagnosis, and systems biology by generating large volume of data for robust analysis. Various components of omics technology are as follows:

3.2.1. Genomics

Genomics and epigenomics emerged as two related but distinct fields of omics. Genomics is the study of an organism's entire genome (complete DNA sequence), identify and understand the functions of specific gene, and analyze overall structure and organization of genome. A DNA sequencing can determine the order of the four nucleotide bases (adenine, guanine, cytosine, and thymine) in a DNA, which is supported by bioinformatics and microarrays (gene expression analysis). Earlier, molecular biology techniques like Northern blot or Real-Time Polymerase Chain Reaction (RT-PCR) were commonly used to detect and quantify individual gene expression [32,33]. Subsequently, TaqMan assay was developed for sensitive and selective quantitative assay of specific nucleic acid sequences by PCR. TaqMan can be used for the detection of nucleotide polymorphisms and other genotoxic endpoints, but not gene function [34,35]. Thereafter, chip-based assays like DNA microarray technology was developed that made appreciable impact in the field of molecular biology and toxicology. These arrays simultaneously measure mRNA levels of thousands of genes in a cell and are capable of detecting even subtle changes in gene expression [35,36]. Genomics study is crucial for understanding an organism's genetic makeup, and has applications in molecular genetics, genome sequencing, disease diagnosis, evolutionary biology, and biotechnology [36-41]. Epigenomics is the study of the epigenome, the complete set of chemical modifications (like DNA methylation, and histone tags) that sit on top of the genome. Such chemical modifications to the DNA affect gene expression, without altering the DNA sequence, cellular processes, and development. These aberrations are often caused by environmental factors, and play crucial role in development, cell differentiation, and disease onset. Epigenomic studies can help identify potential targets for disease prevention and treatment [24-26,37].

3.2.2. Transcriptomics

Transcriptomics studies an organism's transcriptome, which is the complete set of all its RNA transcripts (mRNA, tRNA, rRNA, etc.) in cells. Transcriptomics can identify alterations in gene expression (biomarker) following toxic insult or disease like cancer [24-26]. Identification and characterization of such biomarkers can aid in the diagnosis of poisoning, particularly in ascertaining forensic findings [31]. Various technologies associated with transcriptomics are RNA sequencer, microarrays, single-cell RNA sequencer, and RT-PCR/qPCR (quantitative PCR) [39,41].

3.2.3. Proteomics

Proteomics includes the study of structure and function of proteins, and identify changes in protein expression after toxin insult. Many proteomic technologies can quantify proteins or perform separation of proteins [42,43]. The technologies include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional SDS-PAGE (2D-PAGE), column chromatography like ion-exchange chromatography (IEC), gel filtration chromatography, affinity chromatography, and high-pressure liquid chromatography (HPLC). Combining a protein separation technique like liquid chromatography (LC) with mass spectrometry (MS) can further improve protein identification. LC-MS based analysis of complex mixtures can address several drawbacks with gel-based methods [44-46]. A shotgun proteomic technique, such as isobaric tags for relative and absolute quantitation (iTRAQ) is yet another promising method that involves isotopic labelling of peptides for simultaneous identification and quantification of proteins in a mixture. The labelled peptides are separated by 2D-LC, and analyzed by tandem mass spectrometry (MS/MS). The immobilized metal affinity chromatography (IMAC) can also be used prior to MS analysis [24-26,44-48].

3.2.4. Metabolomics

Metabolomics is the study of metabolites, which are small molecule substrates, intermediates, and products of cell metabolism [24-26,49]. Metabolomic changes occur due to interaction between genetic, environmental, and other factors. Metabolome consists of metabolites (small molecules) that are involved in energy transmission, or cell metabolism [50-53]. The technique used in metabolomics involves MS and nuclear magnetic resonance (NMR) to analyze samples like urine, saliva, or plasma with high accuracy and sensitivity. Gas chromatography (GC) and LC are often used in together with MS to separate metabolites before analysis [51-53]. Metabolomics can be utilized for disease diagnosis, surveillance, drug discovery, development of personalized medicine, nutrition and food science, and understanding gut microbiome [52,53].

3.3. Organ-on-a Chip and Microfluidics Models

Organ-on-a-chip microfluidics refers to the use of miniaturized multi-channel microfluidic devices (micro-physiological systems) with integrated circuits to create a 3D mimic of human organs that offer more human-relevant toxicity data compared to traditional models. These devices house engineered or natural miniature tissues grown inside microfluidic chips that can visualize normal physiological functions and responses to chemicals in a biological system. [54-59]. These devices are sealed, fully sterile, and made of an optically transparent biocompatible polymer material that allows visual and real-time monitoring and analysis of the cell functions. Emulate's liver-on-a chip technology has been used to assess hepatotoxicity and ADME studies of various drugs and chemicals. Other models include gut-on-a-chip, heart-on-a chip, lung-on-chip, and other multi-organ systems [59-61]. Cell clusters using stem cells and induced pluripotent stem (iPS) cells to create organoid have also revolutionized toxicity testing by bridging the gap between cell cultures and animal testing [23,60,61].

3.4. Three-dimensional (3-D) Bioprinting

This technology is a complex biomedical technique that utilizes living cells, biomaterials, and other biological substances in creating 3-D tissue and organ like structure, that mimics human tissues. This system enhances the suitability and reliability of toxicity and safety testing of human relevance [62]. Various 3-D bio-printed models prevalently used include airway models to assess the toxicity and safety of nanoparticles, liver models to study drug metabolism and toxicity, skin models for toxicity and safety of various cosmetic compounds and drugs, and cancer models for efficacy and toxicity of chemotherapeutic drugs [62-64].

3.5. Advanced Imaging Systems

Various advanced imaging systems used in toxicology are non-invasive or minimally invasive techniques that allow spatial visualization of cellular responses to toxicants for studying mechanisms of toxicity, drug development and metabolism, and safety studies [65]. Magnetic resonance imaging (MRI) provides detailed anatomical and functional information like tissue structure, water diffusion, metabolic state of tissues, real-time drug induced lesions and locations, and optimization of tissue sampling for histology [65]. Positron emission tomography (PET) is also a non-invasive imaging technique that uses radioactive tracers for measuring metabolic activity, receptor binding, drug distribution for targeted therapy, and toxicants-induced effects within the living organisms [66-68]. Single-photon emission computed tomography (SPECT) is a nuclear medicine imaging technique that produces 3-D images of various organs and tissues, and obtains anatomical information like tissue density and structural changes through X-ray attenuation [69]. Mass spectrometry imaging (MSI) is a powerful tool in toxicology that allows label-free visualization of drugs, metabolites, and endogenous compounds within tissues. Different MSI techniques like matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and desorption electrospray ionization mass spectrometry imaging (DESI-MSI) are used to analyze various types of molecules and provide insights into drug metabolism and toxicity [65-70].

3.6. Computational modeling

Computational modeling is the application of computers to simulate and study complex systems integrating mathematics, physics, and computer science. It has been widely used across fields like physics, engineering, biology, economics, and social sciences [71]. Computational modeling when applied to biological science is referred as computational biology, that incorporates the techniques of computer science, data analysis, mathematical modeling, and computational simulations to understand the intricacies and interactions of biological systems [72]. Computational toxicology is a class of computational biology where computational tools are integrated into toxicological research for studying toxicity and safety of chemicals, analyses of large and complex data sets, and development of in silico models for predictive toxicology [73]. With the aid of computer models of chemicals and proteins, SARs can be determined by experts in molecular modeling and statistics, who have proficiency in chemistry, biology, and toxicology. Computational toxicology is considered as a suitable alternative to whole animal toxicity testing [71-73]. Among several types of computational models used in toxicology, QSAR model in particular can predict the toxicity of a series of chemicals and identify their potential hazards. Computational toxicology utilizes machine learning (ML) and deep learning (DL)- based algorithms and molecular docking simulations to predict the toxicity potentials of chemicals. The technology mainly aids in forecasting toxicity endpoints and hazard identification, and prioritize chemical testing and safety assessment for regulatory decisions [71-76]

3.7. Other technologies

Advances in toxicology were made possible by development of several smart digital technologies like wearable sensors and mobile technologies, blockchain technology, and advancements in forensic toxicology. Wearable sensors and mobile

technologies have revolutionized toxicology by facilitating continuous and real-time monitoring of individuals experiencing toxic exposure [77]. These technologies use biosensors for early detection of poisoning, improve patient outcome, and obtain data on potential health impacts, and personalized risk assessment. Some of the technologies include wearable sensors, ingestible sensors, wearable electrochemical sensors, head-mounted wearable computers, etc. Some of the wearable technologies may however, have limitations like accuracy, reliability, patient privacy, and security [77,78]. Blockchain is another emerging technology that allows a secure and transparent way to manage and share toxicological data, particularly genomic data related to food safety. It aids in decentralized data keeping and evaluate data that are potential risks to consumers, particularly in areas like clinical trials, pharmacovigilance, and drug safety monitoring, offering potential benefits like improved data integrity and security, transparency, reducing risks of counterfeiting, and more efficient regulatory compliance [78,79]. Technologies with greater sensitivity and specificity are constantly evolving to address the challenges confronted in forensic toxicology. Modern techniques like MS, high-resolution MS, (HRMS), LC-MS/MS, 2-D gas chromatography (2D-GC), surface-enhanced Raman spectroscopy (SERS), Fourier transform infrared (FTIR) spectroscopy, Immunoassay techniques, etc., are being widely employed for detecting specific drugs, chemicals or their metabolites in various biomatrices [80]. Gene editing, especially CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology revolutionized toxicology by allowing precise gene knockout/activation or modifications to delineate chemical toxicity mechanisms, screen for hazardous compounds, model diseases, and create better risk assessment tools [81,82]. Drug development has also experienced crucial role of AI (Artificial Intelligence)- driven clinical trials in compliance with regulatory guidelines [83]. Fig. 1 below summarizes the transition of traditional toxicity testing to new age toxicity testing, and the influence of automated technologies that could produce more human-relevant data for designing regulatory guidelines.

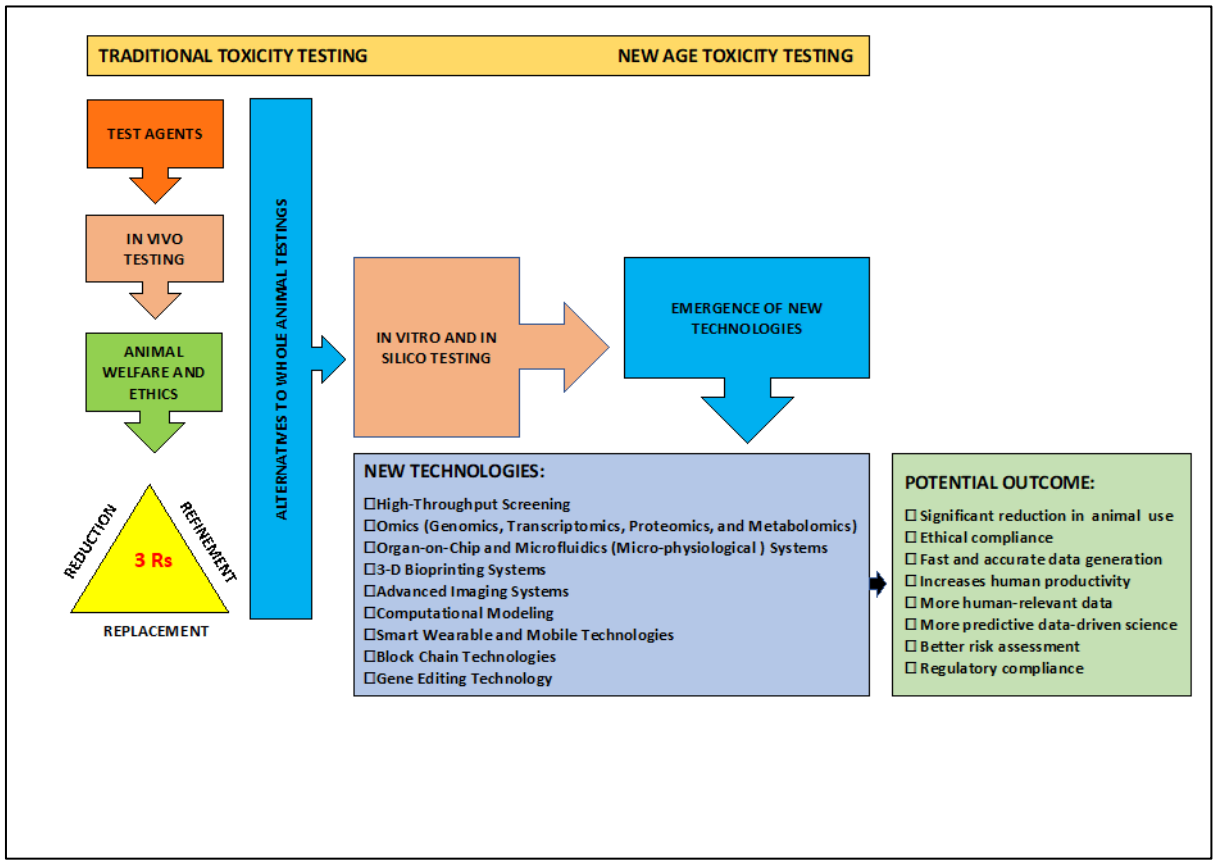

Figure 1 Influence of high-end automated technologies on transition of traditional toxicity testing to new age toxicity testing

4. Conclusion

Integration of emerging tools like HTS, omics technology, and computational modeling have revolutionized the new age toxicology. This enables researchers to understand the mechanism of action of the toxicant, predict its potential hazards, and outline safety guidelines in a more holistic way. A paradigm shift to in vitro and in silico approaches superseded the traditional whole animal testing methods. However, standardization and validation of new technologies need to be

ascertained to a level of regulatory compliance. Also, complexities of real-world exposures involving multiple chemicals and exposure routes need to be addressed. Possibly, reliance on radically new approaches like AI/ML- based computational toxicology, omics technologies, and advanced organ-on-chip models will increase in near future to allow faster, more ethical, and accurate toxicity predictions. There needs to be a mutual trust, transparency, and harmonization of thoughts among the toxicologists, regulatory authorities, and industries in embracing the new technologies to allow creating immense opportunities and challenges for stakeholders across pharma, biotech, and medical technologies.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflicts of interest regarding this article.

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