White Paper

In Vitro vs In Vivo: Advanced Models to Replace Animals

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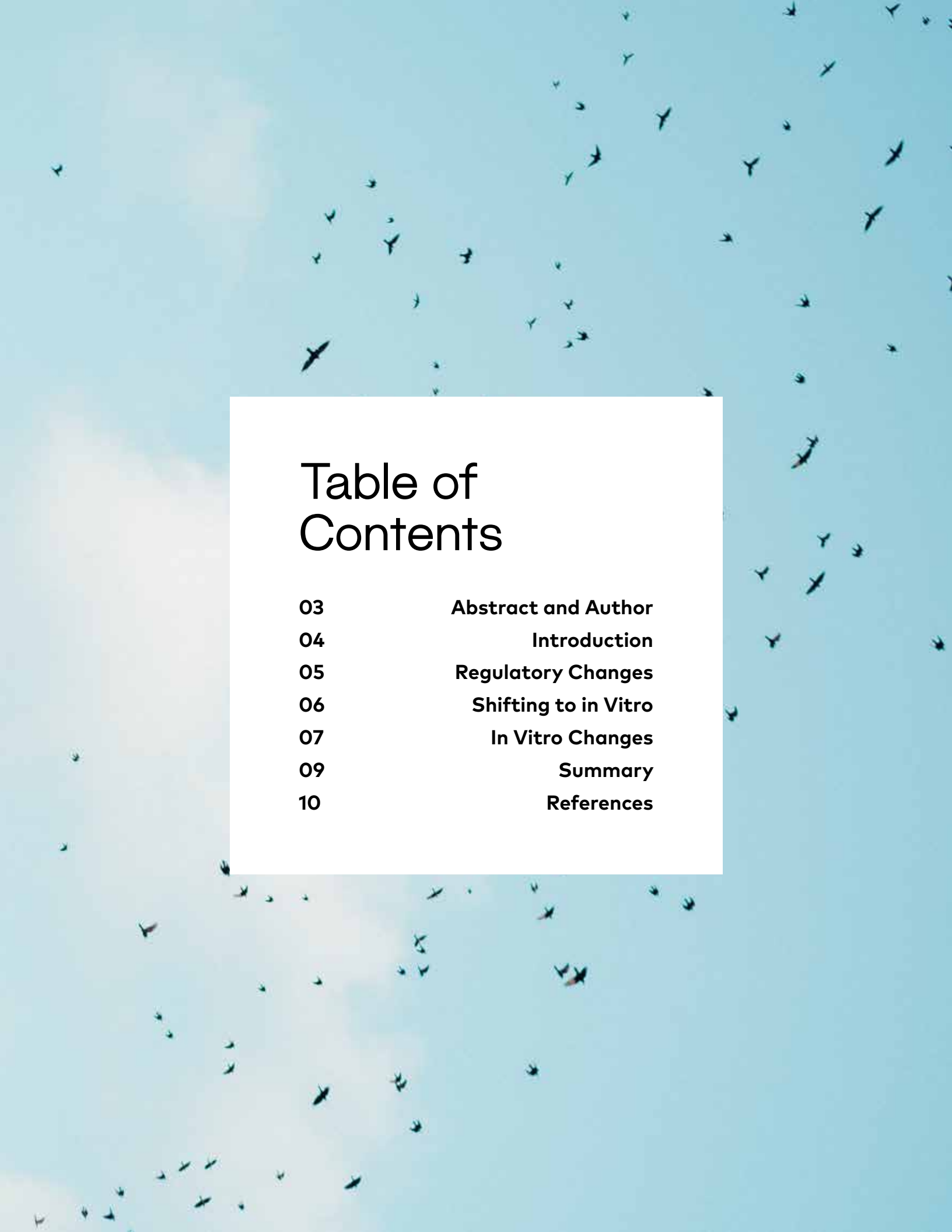

Table of Contents

Abstract

Animal models have been essential for pharmaceutical, small molecule, chemical, and cosmetic development given the inability to test new products directly on humans. However, continued data demonstrates that results from animal studies do not always translate to humans, often resulting in the failure and removal of compounds with limited efficacy or safety concerns during clinical trials or following market release. Research demonstrates the limited translation between models is likely due to the complexity of human physiology which is not fully recapitulated by animal models due to differences in genetic, molecular, immunologic, and cellular responses. Therefore, improved human-relevant models are necessary to truly predict the safety and efficacy of new products prior to market release. This review summarizes current regulatory shifts in the *in vivo* field to *in vitro* alternative methods.

For More Information

Katherine Dunnick, PhD
Author - MatTek Life Sciences

Email: support@mattek.com
Phone: +1 (508) 881-6771

Advanced Models to Replace Animals

Introduction

Animal models are still used for the development of new pharmaceuticals, small molecules, chemicals, and, to a limited degree, cosmetics; however, continued research has demonstrated that animal models do not always provide human-relevant results. The limited translation between animal and human biology results in compounds that can pass safety screenings in animal models but cause adverse reactions in humans during clinical trials or following public release resulting in costly recalls or clinical trial failures. Furthermore, animal models are expensive, time-intensive, have inter-species extrapolation issues, and are low-throughput, making them less ideal for studies. Many of the current in vivo animal tests have also shown poor reproducibility (Costin et al., 2019), further limiting the potential utility and predictive capability of the models.

Researchers have thus turned to human cellular models that can be utilized in high-throughput screening efforts and are less expensive than animal models. Unfortunately, in vitro cell lines grown in monolayer culture fail to replicate the human response due to the limited complexity of a single cell versus a complex organ system. Efforts are underway to develop more complex in vitro systems, including spheroids, organoids, co-cultures, and organ-on-a-chip models. These models aim to increase in vivo relevance in an in vitro system to improve translation to either animal or human models while decreasing costs and increasing efficiency. In addition, they avoid animal welfare concerns and unnecessary pain and suffering to laboratory animals.

Regulatory Changes

Following the release of the "Toxicity Testing in the 21st Century: A Vision and Strategy" report in 2007, the Tox21 federal collaboration was formed in the United States, which sought to develop new methods to rapidly screen compounds for adverse human effects (Tox21, n.d.). In 2013, the European Union finalized a ban on the sale of cosmetics tested on animals, thereby creating a significant need for alternative methods. Since the ban, numerous countries have passed similar laws, demonstrating a continued shift away from animal models toward more advanced, human relevant in vitro models. The US National Research Council has also recommended that animal models be replaced by alternative methods as soon as possible (Mak et al., 2014). Most recently in the United States, the FDA Modernization Act of 2021 was introduced into the Senate, which would authorize the FDA to accept human-relevant testing for efficacy and safety assessment for drug development, shifting away from an 80-year-old requirement that animal tests be used for all new drug applications (Center for Contemporary Sciences, n.d.; H.R.2565 - 117th Congress (2021-2022): FDA Modernization Act of 2021 | Congress.Gov | Library of Congress, n.d.).

These shifts toward more human relevant in vitro models are driven by many factors, including continued failures of compounds in clinical trials, an understanding that animals and humans are not of the same biology, and animal safety and welfare standards. For example, during the drug development process, only 5-10% of compounds that enter clinical trials go on to be approved. The remaining ~90% fail for various reasons from toxicity, limited or no efficacy, off-target effects, and problematic dosing (Hay et al., 2014). This high failure rate indicates preclinical data does not translate well to humans and thus improved methods are needed. For example, TGN1412, an immunomodulatory monoclonal antibody developed to treated multiple sclerosis, rheumatoid arthritis, and certain cancers, was tested on various animal species demonstrating safety and efficacy. Clinical trials that used doses 500 times lower than those administered in animals with no toxic effects, resulted in system organ failure in humans (Mak et al., 2014). While there are numerous case studies demonstrating the limited translation between animals and humans (TGN1412, Vioxx, CEP-1347, IPI-926, etc), animal models are still heavily used for safety, toxicity, and efficacy. For example, although the morphology of rabbit vaginal tissue does not represent human tissue in vivo, personal lubricants and other feminine care products are still tested in a battery of rabbit in vivo tests, including the in vivo rabbit vaginal irritation test. A viable alternative exists in 3D in vitro tissues, which are shown to better mimic human in vivo vaginal tissue, thus providing results more relevant to human end users of the products. (Costin et al., 2019). Furthermore, improved 3D in vitro tissue models have been created to model the human gastrointestinal (GI) tract; however, animal models, which are known to poorly predict GI adverse clinical events, are still relied on for pharmaceutical development (Peters et al., 2019). A shift for alternative in vitro methods is therefore underway and the use of these models is rapidly increasing in the pharmaceutical, cosmetic, chemical, and personal care products industries. In fact, in 2020, the FDA released guidelines that the long accepted local lymph node (mouse) assay used to assess the sensitization potential of topical products was no longer necessary and provided the recommendation that alternative cell-based methods with adequate data to demonstrate predictive human skin sensitization response can be used (FDA, 2020).

Shifting to In Vitro

The most common cause of clinical trial failures is efficacy (54%) and safety (17%) (Fogel, 2018). The current animal models used in preclinical studies to predict efficacy and safety often cannot recapitulate an entire disease and are believed to be the cause for many compounds that fail (Roemer et al., 2014). While animal models are still heavily relied on, continued research demonstrates differences between animal models and humans. Currently, most animal studies utilize mouse models, which are often used to explore new therapeutic approaches and determine the potential success of new drug candidates. While mouse models are heavily utilized, research has demonstrated poor correlation between mice and humans due to differences in cellular responses (Harper et al., 2018; Mak et al., 2014; Seok et al., 2013). Reviews of pre-clinical animal models have reported that only 1/3 of the animal model results translated to humans in clinical studies (Bart van der Worp et al., 2010). These failures, often a result of the animal model not being appropriate for the specific disease due to a lack of disease mechanism understanding, are expensive in terms of both time and money for the pharmaceutical industry.

Given the high percentage of clinical failures, a shift in understanding how chemicals and pharmaceuticals impact humans is underway in the form of alternative in vitro tests. Demonstrating in vivo relevance of these alternative models is vital to shift from animal to human based in vitro pre-clinical assessments. A large shift in the use of in vitro methods has been noted in the ADME, genotoxicity, and safety pharmacology fields, potentially due to the highly validated models with guidelines for use in those fields, such as the in vitro micronucleus test (OECD TG487), Human recombinant estrogen receptor in vitro assay (OECD TG 493), skin irritation (OECD TG 439), eye irritation (OECD TG 492), and skin absorption (OECD TG 428) tests (Jen-Yin Goh et al., 2015). These testing guidelines, along with numerous additional OECD TG, demonstrate improvements in in vitro alternative methods that utilize more human-relevant cells to predict pre-clinical safety.

In Vivo Replacements

Given regulatory guidance to develop in vitro alternative methods, researchers have focused efforts on developing models that utilize human cells in 2D and 3D models to provide improved human-relevance and decreased animal usage. The below are only a subset of approved in vitro alternative methods.

Eye Irritation Test

The Draize test is used to predict irritancy or corrosivity potential in humans for cosmetics, industrial, and pharmaceutical products using animals (Schafer & Bolon, 2017). Recent opposition to this test and data demonstrating lack of objective quantification has resulted in the push to utilize in vitro alternative methods (McNamee et al., 2009). OECD TG 492 utilizes reconstructed human cornea-like epithelium to mimic physiological properties of the human eye. Unlike the animal models that require quantification based on chemosis, ocular discharge, iris abnormalities, etc., the in vitro method measures viability of the in vitro cornea-like epithelium following exposure. If viability remains above 60% following treatment, the test compound can be classified as a non-irritant (McNamee et al., 2009; Starkey, 2012). While this alternative model provides a viable option, continued efforts are necessary to ensure its use throughout industry.

Skin Irritation/ Corrosion Test

The Draize skin irritation test, used to predict chemicals that cause skin reactions, uses rabbits for predicting human responses. As with many in vivo tests, the accuracy of the Draize skin irritation test has been questioned following misclassification of chemicals (Macfarlane et al., 2009). As an alternative, OECD TG 439 utilizes reconstructed human epidermis to identify irritant chemicals. Similar to OECD TG 492, the epidermis is treated with a test materials, compound, or final formulation and the tissue viability is assessed using an MTT assay. This assay is also used to test irritant potential of medical device extracts (ISO - ISO 10993-23:2021 - Biological Evaluation of Medical Devices — Part 23: Tests for Irritation, n.d.). Following treatment, compounds that cause less than 50% cell death are classified as a non-irritant. The reconstructed human epidermis has been accepted by regulatory agencies to act as a stand-alone testing method (Starkey, 2012).

Skin Corrosion Test

Like the irritation test, rabbits are used to test highly reactive, aggressive substances for their skin corrosion potential. The results can be highly variable and are often poor predictors of human reactions (Rooney et al., 2021). In 2002, ICCVAM recommended the use of human skin models as a replacement for animal use in the corrosivity test, and these guidelines were accepted via OECD test guidelines in 2004. OECD TG 431 recommends use of reconstructed 3D human skin that is comprised of an epidermis and functional stratum corneum. Compounds are applied to the human skin and viability is assessed via MTT to classify corrosive or non-corrosive chemicals. This test method can serve as a stand-alone test method for distinguishing severe and less severe corrosives. Additionally, OECD TG 430, which uses rat skin discs to identify corrosives through the assessment of stratum corneum integrity and barrier function changes, and OECD TG 435, which uses artificial membranes to test barrier function, can also be used to test corrosive potential of chemicals (Starkey, 2012).

These three methods represent a small subset of approved, accepted in vitro methods that can be used for assessment of compounds; however, they demonstrate the feasibility to create viable alternative methods that have improved human relevance while also providing accurate predictive results.

Summary

While animal models are still heavily relied on in the field of research, continued efforts are underway for improved in vitro alternative models and methods. With improved understanding of human diseases and the underlying molecular mechanisms, appropriate in vitro models which recapitulate the complexity of these diseases can be developed. Even with OECD approval and regulatory acceptance of alternative models, animals are still used to assess various endpoints that can be detected in vitro. In vitro alternative methods offer many advantages over animal models: they can be utilized in medium- and high-throughput studies, are less expensive, do not have ethical concerns associated with animal use, and can be specifically designed for endpoints of interest. Continued research and regulatory efforts are necessary to implement new alternative methods utilizing advanced in vitro platforms (3D tissues, spheroids, organoids, organ-on-a-chip, etc.) that recapitulate human physiology and disease states better than animal models.

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