Exploration of BioMat-X

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Engineering the microenvironment: advanced biomaterials for humanized in vitro immunotoxicology and carcinogenicity assessment

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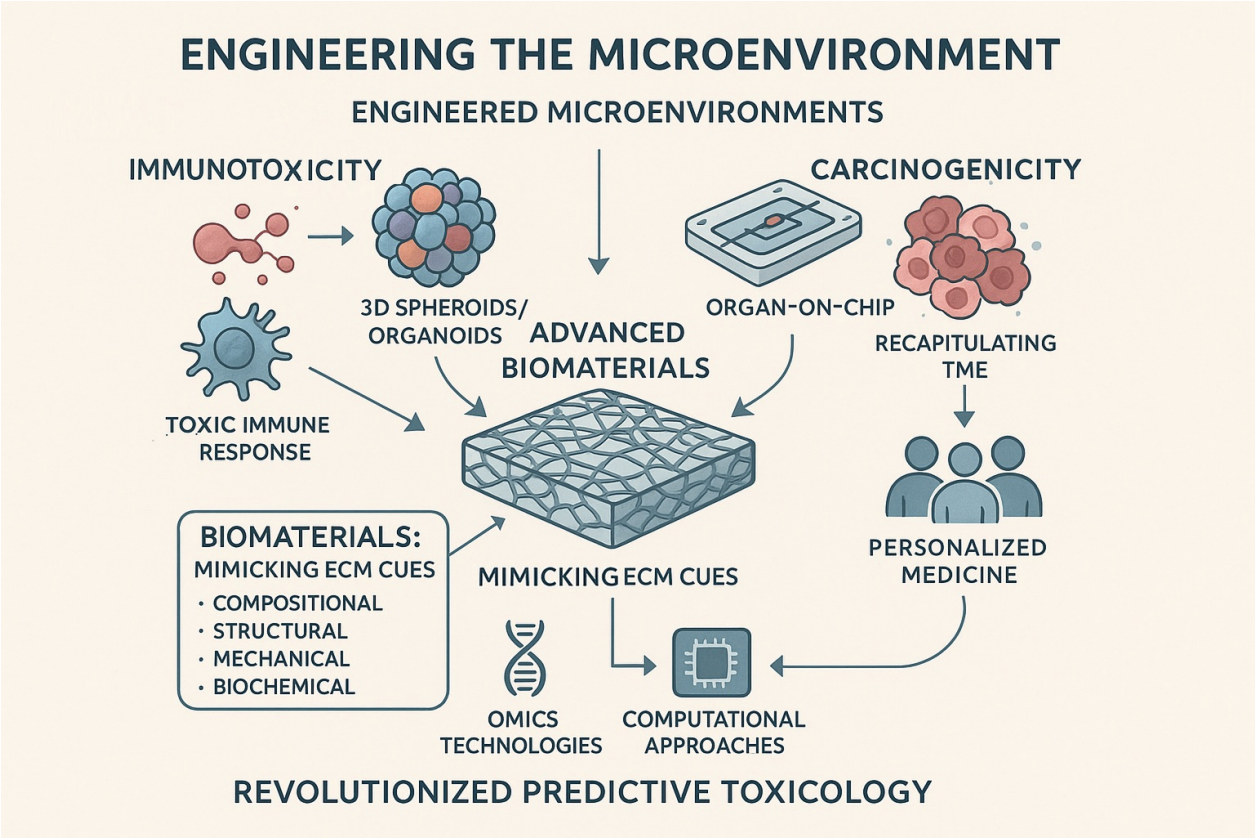
Abstract

The challenges of conventional animal models and two-dimensional (2D) in vitro cell cultures in effectively forecasting human toxicity have prompted a significant shift towards New Approach Methodologies (NAMs). This development centers on advanced humanized in vitro co-culture models that offer improved physiological relevance for toxicological assessment. This perspective highlights the critical role of biomaterials in the creation of complex microenvironments. This study demonstrates how biomaterials effectively mimic the original extracellular matrix (ECM) through controlled compositional, structural, mechanical, and biochemical signals, thereby enabling the development of sophisticated 3D spheroids, organoids, and Organ-on-Chip systems. These biomaterial-enhanced platforms are essential for precise evaluation of immunotoxicity, as they promote human-specific immune responses and targeted immunomodulation, and for carcinogenicity, as they accurately replicate the tumor microenvironment, affect cancer cell behavior, and enable patient-derived models. Moreover, we underscore the synergistic amalgamation of these biomaterial-based models with omics technologies and computational methodologies (QSAR, AI/ML) for thorough molecular insights and rational design. Despite ongoing challenges in standardization and high-throughput compatibility, the strategic utilization of biomaterials is set to transform predictive toxicology, expedite drug discovery, and promote personalized medicine, thereby diminishing dependence on animal testing and improving human safety.



Graphical abstract. Engineering the micro-environment. Created using Napkin AI.

Keywords

humanized in vitro models, advanced biomaterials, immunotoxicology assessment, carcinogenicity testing, 3D cell culture, Organ-on-Chip

Introduction

The assessment of chemical and pharmaceutical toxicity has historically depended on animal models and two-dimensional (2D) in vitro cultures. Although these methods have significantly advanced our understanding of toxicology, they frequently do not provide accurate predictions of human-specific responses. This results in considerable translational gaps, raises ethical issues, and imposes financial burdens due to elevated drug attrition rates [1, 2]. The ethical obligations represented by the 3Rs principle (Replacement, Reduction, and Refinement of animal use), alongside the significant expenses and inefficiencies associated with existing drug development processes, have hastened the transition to New Approach Methodologies (NAMs) [3]. NAMs, characterized by their innovative, human-relevant, and animal-free methodologies for safety assessment, are gaining recognition for their crucial role in enhancing predictive accuracy, ensuring patient safety, and facilitating regulatory acceptance [4].

A significant obstacle in the application of NAMs is their capacity to accurately reflect the intricacies of the human physiological microenvironment. In this context, biomaterials serve a crucial function, as their chemical composition, surface chemistry, stiffness, porosity, and biofunctionalization offer the essential signals needed to replicate tissue-specific architecture, immune cell niches, and tumor microenvironments (TME) [5–7]. The properties in question play a pivotal role in essential cellular processes, including adhesion, migration, differentiation, and immune modulation. These factors directly influence the reliability and translational value of in vitro toxicity assays [8]. The systematic discussion of how biomaterial design parameters impact immunotoxicology and carcinogenicity evaluation is, despite its significance, still insufficiently explored in the literature.

This review explores the pivotal function of biomaterials in the development of sophisticated three-dimensional (3D) in vitro models, such as spheroids, organoids [9], and Organ-on-Chip (OoC) systems. We examine how customized biomaterial systems facilitate the development of physiologically relevant co-culture models for investigating both immunotoxicity and tumor biology, emphasizing recent progress that incorporates mechanical, biochemical, and immunological signals to mimic native tissue function. Furthermore, we investigate the developing collaboration between biomaterials and computational tools, such as omics technologies and in silico modeling [10], as a forward-thinking approach to enhance drug discovery and personalized medicine in a cost-efficient and ethically responsible way [11, 12]. This review explores the intersection of material science and biology relevant to humans, emphasizing both the opportunities and challenges in utilizing biomaterials as the foundational element for advanced NAMs in the assessment of immunotoxicity and carcinogenicity. This paradigm shift from conventional 2D cultures and animal models to advanced biomaterial-enabled 3D humanized systems is illustrated in Figure 1.

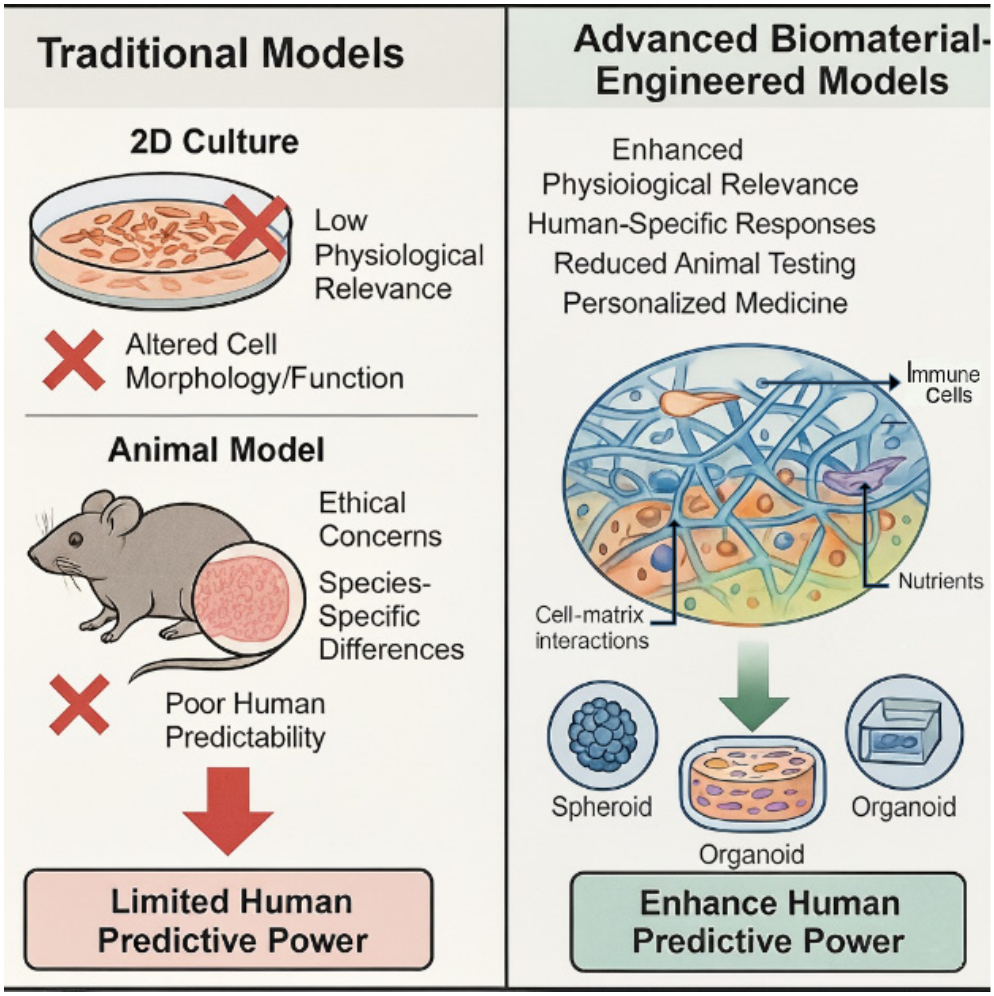


Figure 1. The paradigm shift in toxicology assessment. A comparative illustration highlighting the limitations of traditional 2D cell cultures and animal models in toxicology assessment (low human predictability, ethical concerns) vs. the advantages of advanced 3D humanized co-culture models enabled by biomaterials (enhanced physiological relevance, human-specific responses, reduced animal testing). This shift represents a critical advancement in New Approach Methodologies (NAMs). Created using Napkin AI.

The transformative change in toxicology evaluation

Constraints of conventional animal and two-dimensional in vitro models

For many years, the assessment of chemical and pharmaceutical safety has largely relied on traditional animal models and conventional 2D in vitro cell cultures [13]. While these methods are essential, their inherent limitations have become more evident, prompting a thorough reevaluation of their ability to forecast human health [14].

Traditional animal models present several notable limitations, often considered the gold standard. The 2-year rodent bioassay exemplifies a study that requires significant resources and prolonged observation periods, making it well-known for being time-consuming and expensive [15]. The urgent need to minimize, improve, and replace their use is motivated by significant ethical issues related to animal welfare, as well as practical and financial challenges [16]. Significantly, the predictive accuracy often falls short because of the physiological and immunological differences that exist between animal species and humans [17]. Over 90% of drugs deemed safe and effective in animal studies ultimately do not succeed in human clinical trials, often due to unforeseen toxicity or lack of efficacy. The estimated average cost for developing monoclonal antibodies ranges from $650 to $750 million, highlighting the challenges posed by a high attrition rate, which presents both regulatory issues and substantial financial implications [18]. Specific historical examples that underscore the significant shortcomings in interspecies extrapolation involve the teratogenic effects of thalidomide, which were not foreseen despite extensive animal testing [19]. Furthermore, the immunogenicity observed in animals often fails to align with that in humans, which adds further complexity to safety assessments [20].

While conventional 2D in vitro cell cultures offer cost-effectiveness and high-throughput capabilities, they also possess inherent limitations [21]. Unlike their natural in vivo habitat, cells grown in a monolayer on flat, rigid plastic surfaces exist in an artificial environment. This simplified environment lacks the complex tissue structure, 3D cellular interactions, and crucial physiological gradients (including waste products, nutrients, and oxygen) that are necessary for cellular response and function in a living organism. Consequently, in comparison to in vivo findings [22], results may exaggerate or misrepresent toxic effects, as cells in 2D cultures often exhibit modified morphology, polarity, gene expression, and metabolic profiles [23]. The simplification of biological complexity in 2D in vitro systems, along with the challenges of inter-species extrapolation in animal models, consistently demonstrates that traditional models often fall short in predicting human responses [24]. This synthesis of various issues highlights the intricate nature of toxicology assessment, indicating the need for a fundamental shift in approach rather than minor improvements to existing frameworks [25].

Emergence of new approach methodologies and advances in vitro humanized co-culture models

The deficiencies of conventional approaches have directly driven the urgent advancement and regulatory acceptance of NAMs [26]. These novel methodologies include a wide range of non-animal-based techniques, such as advanced in vitro human-based systems [including cell assays, organoids, and microphysiological systems (MPS)] and in silico (computational) models [4]. The “3Rs principle” provides the fundamental motivation for the development of NAMs, which is to replace, reduce, and refine the use of animals in scientific research and testing [16]. This transformation is not merely a scientific preference; it is a strategic business and ethical imperative that has the potential to revolutionise the entire drug development landscape [27]. These sophisticated models have the potential to significantly reduce research and development costs, accelerate timelines, and enhance predictive accuracy [28]. The schematic representation of biomaterial-assisted models for immunotoxicology and carcinogenicity assessment is shown in Figure 2.

The indispensable role of biomaterials

In order to successfully build physiologically appropriate 3D microenvironments that closely resemble the original extracellular matrix (ECM) and facilitate intricate cellular interactions, these sophisticated in vitro models must be developed [29]. Biomaterials are the basic components that offer structural support, biological signals, and mechanical qualities to these humanised systems [30]. The urgent development and regulatory push for NAMs is directly driven by the significant limits of conventional approaches, and biomaterials are emerging as a key enabling technology for designing the required physiological complexity and human relevance in these novel models [5]. Because of this causal link, human-relevant NAMs are required when standard models fail, and biomaterials are crucial for creating these sophisticated models. This shows how the use of biomaterial-enhanced NAMs is changing the whole drug development landscape and is not only a scientific preference but also a strategic commercial and ethical requirement.

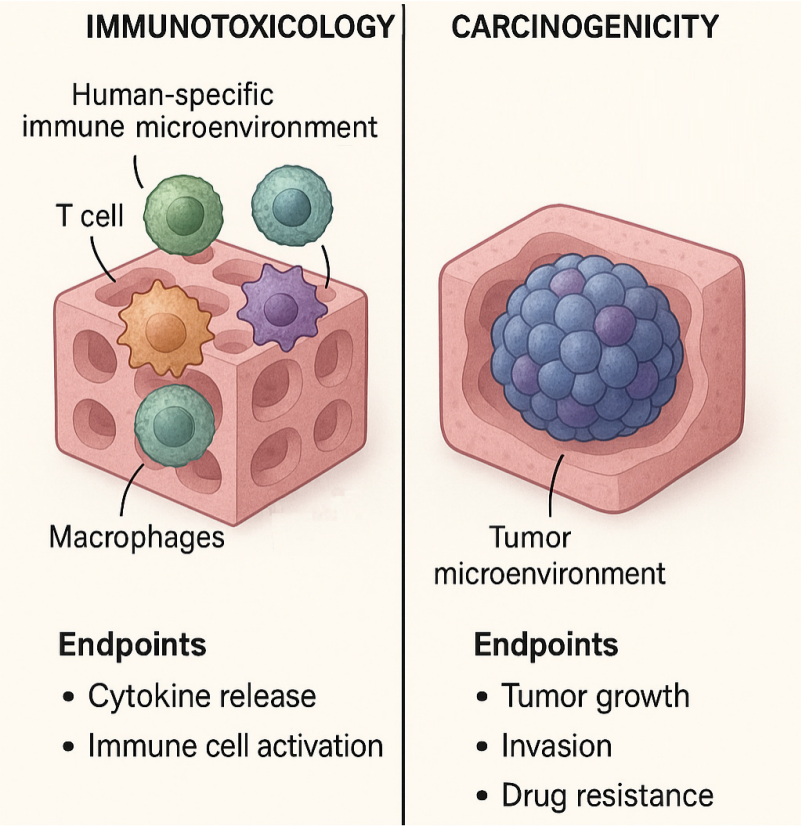


Figure 2. Biomaterial-enhanced 3D models for immunotoxicology and carcinogenicity. Dual-effect showcasing the application of biomaterial-enhanced 3D models in toxicology. **Immunotoxicology:** Depicts immune cells interacting within a 3D biomaterial scaffold, mimicking the human-specific immune microenvironment for assessing immune responses (e.g., cytokine release, cell activation). **Carcinogenicity:** Illustrates biomaterial-assisted tumor models (spheroids, organoids) recapitulating the tumor microenvironment (TME) to study cancer cell behavior, growth, and drug resistance. Both panels emphasize the value of patient-derived models. Created using Napkin AI.

Advanced in vitro humanized co-culture models: mimicking in vivo complexity

By offering a more physiologically pertinent microenvironment, advanced 3D co-culture models, such as spheroids [31], organoids [32], and OoC systems [33], collectively represent a substantial improvement over 2D cultures. This improved realism is essential for the accurate and predictive assessment of toxicology [34].

Overview of 3D cell culture systems

The transition from 2D to 3D cell culture systems has been instrumental in the development of in vitro models that are more physiologically pertinent [22]. These cutting-edge systems are designed to replicate the intricate cellular and tissue architecture that is present in vivo, thereby providing a more precise substrate for the investigation of drug responses and disease mechanisms [22]. A comparative overview of conventional and advanced models used for toxicology and carcinogenicity assessment is provided in Table 1.

The relationship between physiological relevance and screening throughput across various 3D models is summarized in Figure 3.

Spheroids

These are microsized aggregates of closely-packed cells that comprise a fundamental 3D model in cancer research due to their simplicity [31].

Spheroids can be homotypic, which means they are made up of only one type of cell, or heterotypic, which means they are made up of more than one type of cell, including immune cells, fibroblasts, or endothelial cells [35]. The straightforward production and handling of spheroids make them suitable for

Table 1. Comparative analysis of models for toxicology and carcinogenicity assessment.

Feature	Traditional 2D cultures	Animal models	Advanced 3D biomaterial-based humanized models
Physiological relevance	**Low:** lacks tissue architecture, ECM, gradients, and complex cell-cell interactions.	**Moderate:** has systemic physiology but suffers from critical species-specific differences.	**High:** precisely engineered ECM, 3D architecture, co-cultures, and physiological gradients mimic human tissue niches.
Predictive power for human response	**Poor:** high false positive/negative rates due to altered cell states.	**Variable:** often poor, as evidenced by > 90% clinical attrition rate for drugs safe in animals.	**Superior:** human-derived cells in a human-like microenvironment yield more clinically translatable data on efficacy and toxicity.
Immunological relevance	**Limited:** cannot model complex human immune responses (e.g., TDAR).	**Limited:** fundamental differences in immune system function and antigen presentation.	**High:** enables co-culture of human immune and tissue-specific cells to model immunotoxicity, cytokine release, and immunotherapy efficacy.
Throughput & cost	**High:** cheap, scalable, amenable to HTS.	**Very low:** extremely costly, time-consuming, low-throughput.	**Moderate-improving:** higher cost than 2D, but throughput is increasing with automation and standardized biomaterial platforms.
Ethical considerations	**Low concern.**	**Major concern:** significant ethical burden and regulatory push for reduction (3Rs).	**Low concern:** human-centric, reduces reliance on animal testing.
Personalization potential	**Limited:** primarily uses immortalized cell lines.	**None:** Uses genetically homogeneous animal cohorts.	**High:** patient-derived cells [e.g., patient-derived organoids (PDOs)] can be used to create personalized avatars for drug screening.
Key limitation	Over-simplification leads to poor predictability.	Species differences lead to poor predictability and ethical issues.	Standardization, characterization, and integration into regulatory workflows are ongoing challenges.

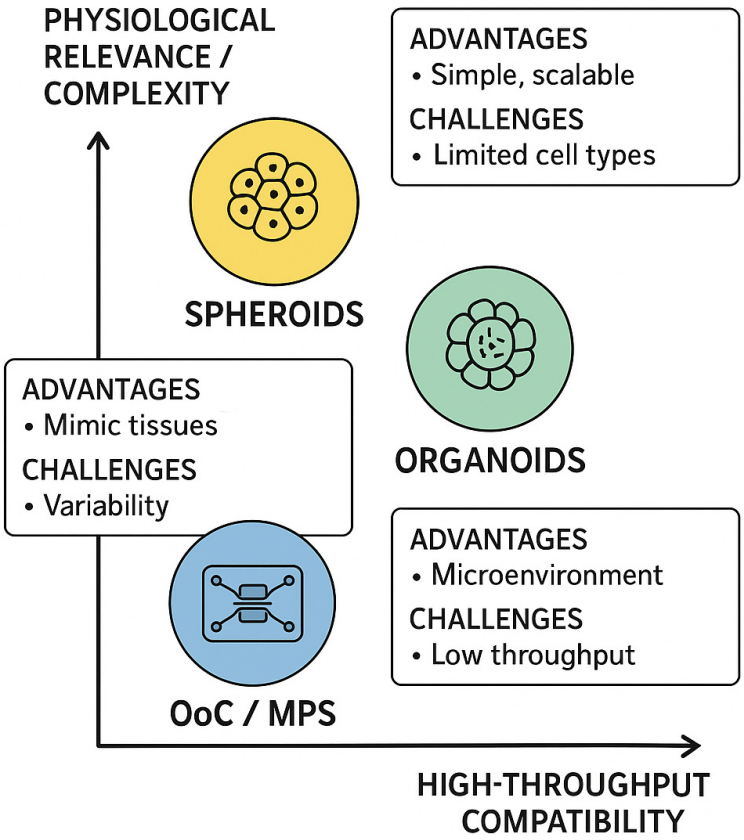


Figure 3. The continuum of 3D in vitro models: complexity vs. throughput. Conceptual diagram illustrating the trade-off between physiological relevance/complexity and high-throughput compatibility across different 3D in vitro models. The figure positions spheroids, organoids, and Organ-on-Chip (OoC)/microphysiological systems (MPS) relative to these parameters, highlighting their respective advantages and challenges in toxicology assessment and drug screening. Created using Napkin AI.

high-throughput screening (HTS) when they come from established cell lines [36]. They effectively mimic or successfully recapitulate important in vivo features, such as creating nutrient and oxygen gradients and forming separate cell layers-a growing outer layer, a resting middle layer, and a hypoxic/necrotic core-which makes them more resistant to drugs than 2D cultures [37]. This layered structure is important for reliable investigations of medication penetration and effectiveness because it mimics the problems that drugs experience in solid tumours in living organisms [38].

Organoids

Organoids are 3D cell cultures that self-assemble into miniaturized organs. They are derived from various stem cell sources, including embryonic stem cells, induced pluripotent stem cells (iPSCs), adult somatic stem cells, or primary tissues [32, 39]. They precisely replicate the structure, function, and cellular diversity of natural organs, encompassing the intricate variations observed in tumors [40]. Patient-derived organoids (PDOs) are effective instruments for personalized treatment, as they retain the functional, genetic, and intrinsic variations among tumors. This capability enables clinicians to predict the efficacy of therapies and the potential for resistance in individual patients. The capacity for personalization is a critical element in the development of advanced in vitro models. The study emphasizes the development of patient-derived systems capable of capturing and predicting individual responses to treatment and the progression of their conditions [41].

Organ-on-Chip/microphysiological systems

OoC platforms signify a significant advancement by integrating dynamic fluid flow and mechanical forces, such as cyclic strain in lung chips and shear stress in vascular channels, into 3D cultures [42]. These systems are generally constructed from polydimethylsiloxane (PDMS) or alternative polymers and feature microfluidic channels populated with living cells. OoCs provide a significant advantage by effectively modeling interactions between multiple tissues and their systemic effects. A liver-tumor-on-a-chip model can effectively simulate the first-pass metabolism of a prodrug and its subsequent impact on cancer cells [43]. Linking an immune component, such as a synthetic lymph node, to a tissue model facilitates the investigation of immune cell trafficking and systemic immunotoxicity, which static models are unable to achieve [44]. Although currently less suitable for HTS compared to spheroids, advancements in automation and multiplexing are swiftly mitigating this limitation.

Importance of co-culture models in simulating complex cell-cell and cell-matrix interactions

Co-culture models are indispensable for recapitulating the complex interplay between different cell populations and the ECM [45]. These assays, which permit the concurrent cultivation of multiple cell types (e.g., immune cells and cancer cells, fibroblasts and epithelial cells), are critical for investigating intercellular signaling pathways [46]. Such systems are fundamental to understanding the TME, a dynamic ecosystem defined by reciprocal communication between neoplastic cells, stromal support cells, and immune infiltrates [47]. Consequently, they provide a more physiologically relevant context than monoculture approaches, where cells are grown in isolation.

Current applications and capabilities

The sophisticated in vitro models mentioned earlier have extensive uses in toxicology, especially in immunotoxicology and carcinogenicity evaluation [7].

Immunotoxicology assessment

Sophisticated co-culture methodologies are being increasingly implemented to evaluate adverse immunological outcomes, including immunosuppression, immunostimulation, and hypersensitivity [48]. These models enable the assessment of critical immune cell parameters, such as proliferation, activation status, and viability. For example, the release of cytokines in response to immunomodulatory compounds can be quantified through assays that utilize human whole blood [49]. Additionally, the impact on cellular integrity or growth can be determined through assessments of specific lymphocyte populations.

Furthermore, sophisticated 3D models that are designed to resemble lymphoid structures are being refined to study intricate immune phenomena, including T-cell-dependent antibody responses [50]. By incorporating tissue models (e.g., liver, tumor) with an immune component, multi-organ-on-chip systems can elucidate systemic drug effects [51]. This allows for the simultaneous study of on-target efficacy and off-target toxicity of cancer therapeutics. The cultivation of cancer spheroids with immune cells in microfluidic devices offers critical insights into the efficacy of immunotherapies and tumor-immune interactions [52].

Evaluation of carcinogenic potential

In addition to the complex interactions between cancer cells and the adjacent TME, these sophisticated models are essential for the investigation of the hallmarks of cancer, such as tumor progression, therapeutic resistance, and metastasis [53]. They are especially beneficial for the differentiation between genotoxic (DNA-damaging) and non-genotoxic carcinogens, which is essential due to the fact that a significant number of carcinogens operate through non-genotoxic mechanisms [54]. Therefore, these systems are being increasingly employed to elucidate the underlying mechanisms of therapeutic resistance and for high-throughput drug screening [55].

Challenges in model development and reproducibility

3D cell cultures continue to face challenges in gaining widespread acceptance, despite their successes. The production of varying findings by different laboratories presents a challenge in terms of comparing work and obtaining regulatory approval, as there is no single best practice for the establishment and maintenance of these cultures [8]. Data collection and verification are difficult due to the extreme complexity of 3D models, which necessitate sophisticated tools and computational models, despite their usefulness for real-world relevance [56]. Furthermore, the utilization of these complex cultures may be restricted, particularly for large-scale initiatives, due to the time and expense necessary to acquire and maintain them. Organoids are highly appropriate, but their variability is a significant challenge for mass screening due to the complexity of their natural detail and the difficulty of generating uniform forms from a limited number of cells [57]. Many organoid settings still lack the comprehensive immune-defense component necessary to comprehensively test novel immune treatments and cancer-immune conflicts [58]. A significant judgment must be made regarding the transition from basic spheroids to more complex systems, such as multi-OoC. Spheroids are renowned for their simplicity and efficacy in mass screening [36], whereas organoids, with their complexity and diverse applications, are difficult to integrate into mass screening due to their fluctuations [57]. This illustrates that 3D models that are more realistic and complex are more costly, more challenging to disseminate, and less suitable for mass screening. Consequently, the decision-making process is complex and contingent upon the study's requirements and stage [59]. A comparative summary of the advantages, limitations, and applicability of these advanced 3D in vitro models is provided in Table 2.

Biomaterials: the foundation of humanized in vitro systems

In human-like laboratory models, the primary function of biomaterials is to replicate the complex combination of chemical and biological characteristics found in the natural ECM [62]. This replication task is indispensable for the behavior, function, and management of illness by cells that are in accordance with reality [63]. Biomaterials are dynamic components that contribute to the ability of synthetic components; they are not merely inert elements [30].

Mimicking the extracellular matrix

Robust filaments create a network in the ECM. It comprises adhesive proteins, substantial proteins, and collagens [62]. The body's cells get crucial signals from this network [30]. Biomaterials are meticulously designed to emulate these complex components. High-level laboratory models offer the requisite shape and function as a framework [5].

Table 2. Comparison of advanced in vitro models for toxicology assessment.

Model type	Physiological relevance (ECM mimicry, cell-cell interactions, gradients, tissue architecture)	Throughput capability (HTS compatibility)	Suitability for immunotoxicology (immune cell integration)	Suitability for carcinogenicity (TME mimicry, drug screening)	Key advantages	Key challenges
Spheroids	**Moderate:** 3D cell-cell interactions, gradients, basic TME mimicry [37, 38].	**High:** especially when using established cell lines [31, 36].	**Good:** heterotypic spheroids can be co-cultured with immune cells [35, 52].	**Good:** models drug resistance, TME mimicry, and proliferation effectively in a 3D context [37, 46].	Simplicity, cost-effectiveness, scalability, HTS compatibility [31, 36].	Lack of complex tissue architecture, limited long-term stability for primary cells, batch-to-batch size variability [8, 35].
Organoids	**High:** self-organization creates organ-specific cell types, complex tissue architecture, and patient heterogeneity [32, 40].	**Moderate:** significant variability in size and shape makes them less amenable to HTS than spheroids [57].	**Moderate:** Some co-culture with immune cells is possible, but they often lack an autologous vascularized immune component [58].	**High:** Patient-derived organoids (PDOs) allow for high-fidelity drug screening and capture the genetic landscape of the original tumor [9, 41].	High human relevance, personalized medicine potential, recapitulates disease complexity [9, 32].	High variability, standardization challenges, difficult integration with HTS, and a lack of a complete immune/stromal microenvironment [8, 57, 58].
Organ-on-Chip (OoC)/microphysiological systems (MPS)	**Very High:** incorporates dynamic fluid flow, physiological mechanical forces, multi-cell co-cultures, and systemic effects [33, 60].	**Moderate to high:** Throughput is improving with automation and standardized formats, but is not yet at traditional HTS levels [57].	**High:** can simulate systemic effects and immune cell trafficking by connecting tissue compartments (e.g., tumor and lymph node) [51, 61].	**High:** allows for dynamic TME emulation, multi-organ interactions (e.g., metabolism-toxicity), and advanced drug screening [33, 51].	Mimics in vivo environment, uses human cells, allows real-time monitoring, enables multi-organ integration [33, 61].	High cost, operational complexity, challenges in standardization and scale-up, complex data analysis [8].

Composite material

The ingredients that are used to create the biomaterial are sourced from both the laboratory and natural sources.

- **Natural polymers:** Collagen [64], fibrin, HA [65], and gelatin [66] are all substances that are well-absorbed by the body, do not induce numerous immunological issues, bind to cells [67], and are degraded with the help of enzymes. These fragments have the potential to transform into polymers that resemble the squishy, moist regions where cells reside in our bodies. This assists cells in maintaining their wrapping and acquiring nutrients.
- **Synthetic Polymers:** PEG [68], PIC, PAAm, and PVA [69] are among the materials that assist us in the manipulation, combination, and production of these components in a highly compact manner. It is possible to modify them to meet specific requirements, as they are intended to prevent the production of inflammation or immune responses.
- **A combination of the two:** By combining natural polymer and synthetic polymers, the advantages of both can be achieved, thereby opening up a wider range of options for adjusting the firmness and chemical activity of the polymers for individual cell growth assays. For example, GelMA [70] integrates the cell clues of gelatin with the ability of synthetic components to form in response to light.

Structural fidelity

The composition of biomaterials affects the behavior of cells [71].

- **Porosity:** The pores in biomaterial grids are just the correct size to allow food to pass through, waste to be eliminated, and cells to adhere to one another to build 3D structures. Cell migration and development are determined by the hole diameters and their connections.
- **Topography:** Small elements such as shapes, lines, or small posts can be included in biomaterials. This facilitates the attachment, growth, migration, and adaptation of cells to the rough surface of native tissue.
- **Fiber alignment:** Using techniques like electrospinning [72], we can create microscopic to enormous fiber patterns that closely resemble real ECM fibers. These lines signal cells where to go, promote EMT, and aid in cell growth. When it comes to the spread of cancer, this is crucial. The ability of engineered biomaterials to reproduce the compositional, structural, and mechanical cues of the native ECM is depicted in Figure 4.

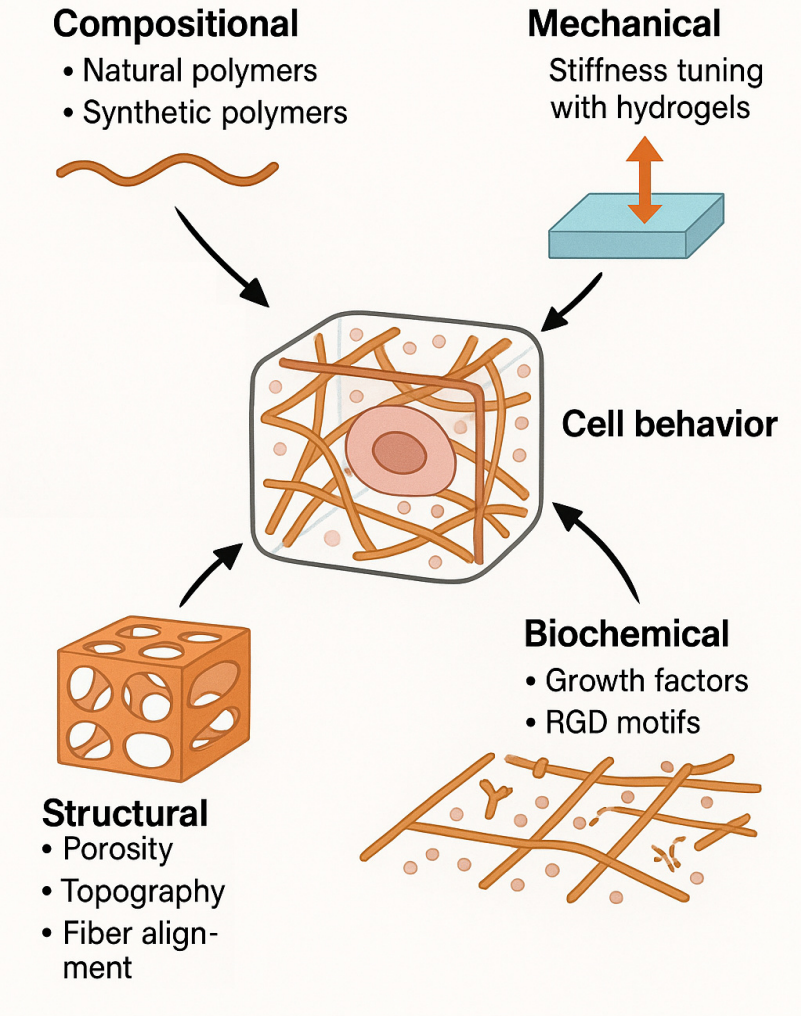


Figure 4. Biomaterial engineering of the ECM microenvironment. Schematic representation detailing how biomaterials precisely mimic the native extracellular matrix (ECM) through controlled compositional, structural, mechanical, and biochemical cues. The figure illustrates examples of biomaterial properties (e.g., polymer types, porosity, stiffness, growth factors) and their influence on cell behavior within a 3D context, crucial for creating physiologically relevant in vitro systems. Created using Napkin AI.

Mechanical fidelity

The ECM's mechanical properties change throughout time and have a big impact on how cells develop and work. Biomaterials are made to copy these mechanical signals [73].

- **Stiffness:** Hydrogels, a common type of biomaterial scaffold, can be tuned across a wide physiological range of stiffness, from extremely soft (mimicking brain tissue at ~0.1 kPa) to highly rigid (mimicking bone at ~10 MPa) [74]. This property is a critical regulator of cancer progression. Matrix stiffness directs cancer cell morphology, epithelial-to-mesenchymal transition (EMT), and the proliferation, invasion, and drug resistance of cancer stem cells. For instance, increasing matrix rigidity can induce irregular spheroid morphologies and promote the widespread dissemination of pancreatic cancer cells [74]. Stiffer microenvironments, particularly when combined with biochemical cues like growth factors, can also drive invasive phenotypes.
- **Dynamic and active properties:** Beyond static stiffness, "active" or "smart" hydrogels can replicate the dynamic changes in ECM mechanics that occur during disease progression, such as wound healing or tumor stiffening [75]. These materials can be designed to soften or stiffen over time, mimicking the constant interaction between cells and their environment. The matrix's topography and its ability to be remodeled by cellular forces are also crucial, as they enable cells to generate traction for invasion, motility, and the formation of branching structures [75].

Control of biomaterial properties for tailored microenvironments

The precise regulation of porosity, topography, and stiffness is paramount for designing biomimetic microenvironments. These parameters are not intrinsic but are actively engineered through specific fabrication techniques and material choices. Control strategies include modulating polymer concentration, crosslinking density (chemical or physical), and employing advanced fabrication methods like electrospinning and 3D bioprinting. The following table summarizes key parameters and methods for controlling these critical properties. The principal parameters and techniques for modulating porosity, topography, and stiffness in biomaterials are outlined in Table 3.

Table 3. Engineering biomaterial properties to control the cellular microenvironment.

Property	How to control it	Key techniques for control	Impact on cell behavior
Porosity	Polymer concentration Crosslinker ratio/type Porogen (e.g., salt, sugar) leaching Gas foaming Freeze-drying (cryogelation)	Electrospinning (fiber spacing) 3D bioprinting (strand deposition) Particulate leaching Solvent casting	**Nutrient/waste diffusion:** high porosity enhances transport. **Cell migration/invasion:** Pore size dictates if cells can infiltrate. **Vascularization:** critical for promoting blood vessel ingrowth.
Topography	Master mold fabrication (lithography) Fiber alignment (electrospinning) Surface patterning Scaffold architecture design	Soft lithography Micro/nano-patterning Electrospinning 3D bioprinting	**Cell alignment:** grooves/fibers guide cell orientation (e.g., neurites, muscle cells). **Focal adhesion:** nanoscale features alter integrin binding and signaling. **Migration:** contact guidance directs cell movement.
Stiffness	Polymer concentration Crosslinking density (UV intensity, time, initiator concentration) Polymer choice (e.g., PEG vs. alginate)	Tunable hydrogels (PAAm, PEGDA) Hybrid systems (e.g., GelMA) Dynamic crosslinking	**Stem cell differentiation:** Soft matrices (~0.1–1 kPa) promote neurogenesis; stiffer matrices (~10 kPa) promote osteogenesis. **Cancer progression:** increased stiffness can promote invasion and EMT. **Cell spreading:** Stiffer substrates typically promote greater cell spreading and traction.

Biochemical cues

In addition to providing physical support, biomaterials deliver specific biochemical signals.

Biomaterials can be designed to contain growth factors (e.g., BMPs, TGF-β, FGF-2, VEGF) and establish small chains (e.g., the RGD sequence) that alter cell function, assist in cell adhesion, growth, and the

formation of new blood vessels [76]. The cells are provided with these clues in a specific manner, which replicates the endogenous signals present in the body. Motifs that are degradable proteases can be incorporated into synthetic biomaterials, enabling a cycle of ECM change and cell movement. This replicates the natural changes in body tissue and enables cells to modify their own environment [75].

Advanced biofabrication techniques

Complex methods of producing biological materials, such as 3D bioprinting [77], are not merely instruments for producing objects. They are inextricably linked to novel biomaterial designs, such as specialized bioinks [78]. This link enables us to oversee the construction and composition of objects at unprecedented levels. Consequently, we can develop more accurate and realistic models for laboratory testing.

Function of 3D bioprinting

3D bioprinting is a fabrication technology that enables the layer-by-layer application of biomaterials (bioinks) and cells to create intricate 3D tissue structures [77]. This technique is ideal for the creation of detailed in vitro models because it provides precise control over the placement of cells, enables the growth of the model, and offers cost savings. Additionally, it displays fine detail. It enables us to create shapes that closely resemble actual body parts and incorporate numerous active components in a manner that is both adjustable and programmable.

Development and properties of bioinks

Bioinks are essential for bioprinting. They are primarily composed of a bio-solution, such as a hydrogel, that encases the desired cells. A bioink that is suitable for specific tissues must be strong, able to set correctly, secure for cells, and degrade efficiently [78]. Bioinks that set under light (photopolymerizable bioinks, PBs) are advantageous because they enable the creation of highly detailed and stable components, the packing of normal cell quantities, the establishment of chemical spans in 3D tumor models, and the creation of channels resembling those found in blood. These PBs are compatible with a variety of bioprinting methods, including extrusion, inkjet, and vat polymerization, which enable us to precisely position cells and construct intricate structures. This combination enables us to create intricate, multi-material, and well-formed micro-environments that were previously impossible to create.

Smart and dynamic biomaterials

Moving from stationary frames to open systems that better adapt to the evolving behaviors of living tissues, the future of biomaterials in toxicological models seeks to create smart and dynamic microenvironments that can act upon and modify biological responses.

Stimuli-responsive biomaterials

These novel materials are designed to respond autonomously to external stimuli (such as light, magnets, or sound waves) or to specific bodily indicators (such as changes in pH, temperature, or the amount of work an enzyme is performing) [79]. They are able to adjust to their little world due to this autonomous responsiveness. In order to make things function as they do in the body with conditions like hypoxic regions within tumors or areas of inflammation, it is crucial to be able to easily set when medications go out or target body defenses. For instance, some microscopic technology may create 3D web-like structures inside physical bodies, interfering with cell connections and preventing the formation and spread of cancer.

Self-healing hydrogels

These hydrogels have the capacity to mend their own cracks, much like nature does [80]. As a result, they have a longer lifespan and are more stable for use in 3D cells and in bodily implants [81]. Some of these hydrogels even have the ability to prevent damage from waste and air, which helps to maintain a healthy and clean cell environment.

Biomaterials that modulate immunity

A novel class of biomaterials is now able to influence the body's defensive response in addition to being safe to utilize with the body [82]. By altering immune cells' surface characteristics (such as how they influence T cell and macrophage kinds) and gradually releasing factors that regulate immunological activity, they help prevent negative immune reactions, repair tissues, and decrease swelling. This is a significant step toward creating well-controlled in-vitro models that possess greater physiological relevance. A concise overview of natural, synthetic, and composite biomaterials and their respective contributions to ECM mimicry is presented in Table 4.

Biomaterial-driven innovations in immunotoxicology assessment

Biomaterials provide a precise and adjustable method for engineering the immune microenvironment within in vitro models, enabling the targeted study and modulation of human-specific immune responses. Consequently, they surpass the substantial constraints of animal models in the prediction of immunotoxicity [92].

Developing biomaterial platforms for human-specific immune responses

Biomaterials are being developed to establish human-specific immune microenvironments in vitro, as animal models are not reliable predictors of human immune responses due to inherent species differences, particularly in immunogenicity to human monoclonal antibodies [20]. This is an essential step towards the development of immunotoxicity assessments that are more human-relevant and reliable.

Biomaterials can be engineered to facilitate the delivery and support of immunomodulatory stromal cells, including fibroblastic reticular cells (FRCs). These cells can enhance immune regulation by influencing T cell activity and promoting tolerogenic phenotypes. For instance, 3D macroporous gelatine scaffolds have been demonstrated to promote the survival of FRCs in vitro, augment the expression of their phenotypic markers, and increase the secretion of anti-inflammatory cytokines such as TGF-β2. These scaffolds resulted in decreased cytotoxic T cell activity and the promotion of regulatory and anergic T cell phenotypes when co-cultured with diabetogenic T cells. These phenotypes are associated with immunological tolerance rather than autoimmune activation. This illustrates the ability of biomaterial architecture to influence the behaviour of FRC and the interactions between immune cells. Additionally, the behaviour of a variety of immune cells, such as macrophages [93], dendritic cells, and T cells, can be actively influenced by biomaterials. By precisely regulating surface properties, including charge, hydrophobicity, and roughness, and by regulating the release of immunomodulatory factors, including anti-inflammatory cytokines, this modulation is accomplished [94]. This represents a shift from the passive observation of immune responses to the engineering of them in a controlled in vitro environment, a significant innovation for the accurate assessment of immunotoxicology.

Regulating immune cell function and behaviour in engineered microenvironments

The interaction between biomaterials and the host immune system is intricate and multifarious, involving dynamic phenotypic shifts, receptor engagements, and intricate signalling pathways (e.g., M1/M2 macrophage polarisation) [93]. This requires the development of advanced biomaterials in order to obtain immunomodulatory results that are both predictable and specific [94].

B and T cell activation, differentiation, and cytokine production can be either stimulated or suppressed by biomaterials through their signalling pathways and transcription factors. Specific immune cell receptors and pathways are influenced by a variety of natural biomaterials, including collagen, gelatin, silk, fibrin, alginate, chitosan, and hyaluronic acid. For instance, collagen influences cell adhesion, proliferation, and inflammatory cytokine production by interacting with integrins, discoidin domain receptors (DDR), and OSCAR. Chitin-binding receptors and TLR-2 can recognise chitosan, which results in the activation of immune cells and the production of pro-inflammatory cytokines such as TNF-α and IL-6. Hyaluronic acid has the potential to influence the polarisation of macrophages towards an anti-inflammatory (M2) phenotype [65].

Table 4. Key biomaterial types and their roles in ECM mimicry.

Biomaterial category	Specific examples	Key properties (biocompatibility, degradability, mechanical tunability, cell adhesion motifs)	Contribution to ECM mimicry (compositional, structural, mechanical, biochemical cues)	Advantages of in vitro models	Associated challenges
Natural polymers	Collagen, fibrin, hyaluronic acid (HA), gelatin, alginate, chitosan, silk fibroin	High biocompatibility [83], low immunogenicity [84], inherent cell adhesion sites (e.g., RGD) [67], enzymatic degradability [66].	**Compositional:** mimic native ECM proteins/polysaccharides [63]. **Structural:** form hydrogels, fibrous networks [64]. **Biochemical:** present cell-binding motifs, sequester growth factors [76].	Excellent biological relevance, support cell growth/differentiation, tunable properties [66].	Variable batch-to-batch consistency, potential for immunogenicity (though low) [84], limited mechanical strength for some [85].
Synthetic polymers	Polyethylene glycol (PEG), polyisocyanide (PIC), polyacrylamide (PAAm), polyvinyl alcohol (PVA)	High tunability in physical/chemical properties, controllable degradation, low immunogenicity, resistance to non-specific protein adsorption [68, 69].	**Structural:** precise control over architecture, porosity. **Mechanical:** tunable stiffness/elasticity [73]. **Biochemical:** functionalizable with specific cues [30].	Greater control over properties, reduced variability, avoids animal-derived components, can be stimuli-responsive [79].	Often lack intrinsic bioactivity/cell adhesion, may require functionalization, potential cytotoxicity from residues [86].
Composite (semi-synthetic) biomaterials	Gelatin methacryloyl (GelMA), HA-PEG composites	Combines bioactivity of natural polymers with tunability/stability of synthetics; photopolymerizable, customizable mechanical properties [62, 87].	Comprehensive mimicry (compositional, structural, mechanical, biochemical) by integrating the best features of both categories [88].	Broad range of adjustable properties, enhanced physiological relevance, improved printability for bioprinting [89].	Balancing properties can be complex, potential for residual toxicity from crosslinking agents, regulatory hurdles for novel combinations [70].
Bioinks (specialized for 3D bioprinting)	GelMA, HAMA, alginate, recombinant spider silk proteins, cell aggregates	Tunable rheological properties (shear-thinning) [90], rapid gelation post-printing, biocompatibility, cell encapsulation capability [78].	Enables precise spatial patterning of cells and ECM components, vascularized channels, biochemical gradients, tissue-mimetic stiffness [77, 91].	Allows for complex, high-resolution 3D tissue constructs, high reproducibility, scalability for HTS [77].	Cytotoxicity due to UV light/free radicals (for photopolymerizable), oxygen inhibition, long printing times, need for more relevant ECM mimics [78].

The surface chemistry (e.g., hydrophilicity, roughness, functional groups) and topography (e.g., pore architecture, size, shape) of biomaterials are essential in determining macrophage activation, polarisation (M1/M2 phenotypes), and overall immune cell migration and infiltration [94]. For example, macrophage activation markers can be upregulated by increased surface roughness, and anti-inflammatory macrophage polarisation can be improved by specific pore architectures. The modulation of immune reactions is also significantly influenced by the mechanical properties of biomaterials, such as stiffness and elasticity, which influence macrophage polarisation and neutrophil migration. Moreover, the immune system can be influenced by the degradation products released from biomaterials, which can affect the macrophage phenotype and the overall inflammatory response. This underscores the dynamic nature of these interactions over time. This level of detail underscores the complexity of the task of designing biomaterials for immunomodulation; it necessitates a profound comprehension of the intricate molecular and cellular interactions in order to accomplish predictable, desired immune responses, rather than merely general "biocompatibility" [83].

Examples of biomaterial-enhanced models for the evaluation of immunosuppression, immunostimulation, and immune-mediated adverse effects

Biomaterial-enhanced 3D models are essential for the development of in vitro assays for previously intractable and complex immunotoxicology endpoints, such as T-cell dependent antibody responses (TDAR) and systemic immunotoxicity [48]. Traditional 2D in vitro methods were unable to capture these endpoints due to their lack of physiological complexity. Peripheral blood mononuclear cells (PBMCs) and other immune cell types are commonly employed in in vitro humanised models to evaluate immunotoxicity, including cytotoxicity, proliferation, and cytokine release, in co-culture with target cells. These assays are capable of detecting any monoclonal antibody that could potentially induce a hazardous cytokine surge, thereby offering a critical safety net that animal tests frequently failed to provide [49]. In order to evaluate vaccine formulations and adjuvants, dendritic cell activation assays are implemented to evaluate innate immune activation.

In order to evaluate intricate immune responses, including TDAR and immune enhancement, engineered lymph node models and other advanced 3D immune MPS are currently being developed [50]. These intricate endpoints necessitate appropriate matrices and spatial organisation of pertinent immunological compartments, which 2D in vitro systems are incapable of delivering. Within a single human microphysiological model, multi-OoC setups can simulate systemic pharmacodynamic effects by connecting a variety of tissue chips (e.g., liver, tumour) with an immune compartment to investigate the effects of immunotherapy, including on-target tumour killing and off-target organ toxicity [51, 95]. The co-culture of cancer spheroids (e.g., MCF7 breast cancer) with T-cells from human PBMCs within microfluidic systems offers a platform for the study of immune cell infiltration and modulation, as well as tumor-immune cell interactions and immunotherapeutic responses [52]. This suggests that biomaterials are directly addressing critical gaps in in vitro immunotoxicology by facilitating complex 3D structures and multi-cellular co-cultures, thereby enabling the study of functional immune responses that rely on tissue-like organisation and cell-cell crosstalk.

Enhanced carcinogenicity assessment with engineered biomaterials

Engineered biomaterials precisely replicate the ECM, which is not merely a passive scaffold but an active and dynamic regulator of critical cancer hallmarks, such as cell morphology, stemness, proliferation, migration, invasion, and drug resistance [95]. The precise control of these aspects in vitro is now possible due to the active function of biomaterials, which enables targeted studies of these complex mechanisms.

Impact of biomaterial properties on cancer cell behaviour

Biomaterial properties, such as their architecture (dimensionality, topography, porosity) and mechanics (stiffness, elasticity, plasticity), significantly influence critical cancer hallmarks [76]. These properties have the potential to substantially influence cell migration, invasion, and drug resistance, as well as to promote EMT, enrich cancer stem cell populations, and modulate proliferation. Additionally, they can direct cancer cell morphology. Mechanical and biochemical signals that are essential for tumour progression can be influenced by 3D scaffolds that are engineered from biomaterials, which can effectively replicate the hypoxic and nutrient-deprived conditions of the in vivo TME [95].

Stiffness

Mechanical stiffness of the biomaterial matrix is a critical regulator of cancer cell behaviour [96]. The dynamic gelatin-HA hydrogels exhibit the ability to induce highly irregular spheroids and the extensive dissemination of pancreatic ductal adenocarcinoma (PDAC) cells by increasing the matrix rigidity from approximately 1 kPa to 3.5 kPa. In cancer cells, a mesenchymal phenotype can be induced by stiff matrices, particularly when combined with growth factors such as TGF-β1, which promotes invasiveness [97]. In many cases, the optimal matrix rigidity for CSC growth and marker expression is tissue-dependent, underscoring the necessity of customised biomaterial design. PEG-phosphorylcholine hydrogel studies have also demonstrated that increased substrate stiffness can substantially contribute to drug resistance and enhance breast cancer cell migration.

Architecture

Biomaterials' architectural characteristics, including porosity, topography, and dimensionality, are essential in determining the behaviour of cancer cells. 3D scaffolds have the potential to enrich CSCs, promote EMT, induce invadopodia formation, and influence drug resistance and proliferation [96]. The migration and invasion of cancer cells are significantly facilitated by fibrillar 3D matrices with nano- to micro-porous architectures, which suggests that pore size may be more significant than stiffness in determining invasion efficacy. This progresses from a basic comprehension of biomaterials as structural supports to the recognition of their active role in shaping cancer progression, which biomaterials can now precisely control in vitro, enabling targeted studies of these complex mechanisms to be conducted.

Development of biomaterial scaffolds derived from patients for personalised cancer models

Biomaterials are a critical component of personalised oncology in vitro, as they facilitate the development of patient-derived tumour models that more accurately predict therapeutic responses and faithfully conserve individual tumour heterogeneity than conventional, non-patient-specific models [98]. Grown within biomaterial scaffolds, patient-derived tumour organoids (PDTOs) are becoming potent models for personalised cancer therapy [99]. The functional, genetic, and intrinsic heterogeneity of the original tumour is consistently preserved by these models, rendering them highly appropriate for the identification of resistance mechanisms, the discovery of prognostic biomarkers, and the assessment of patient-specific drugs [41]. Biomaterials facilitate the growth, differentiation, and maintenance of PDTOs by providing the essential scaffold and biochemical cues required for their physiological relevance and the ability to replicate the in vivo TME [5]. The precise construction of biomimetic models using patient-derived tumour cells and their associated ECM is made possible by advanced 3D bioprinting techniques [77]. This approach further advances the potential for truly personalised treatment strategies by enabling the arrangement of cells along complex 3D architectures. Biomaterials facilitate the accurate recapitulation of patient-specific tumour biology, which in turn enables the identification of resistance mechanisms and personalised drug screening. Consequently, a direct causal link is established.

Biomaterial-assisted models for high-throughput drug screening and understanding mechanisms of drug resistance

Biomaterial-assisted 3D models are fundamentally changing the direction of cancer drug discovery from simple efficacy screening to a deeper, mechanism-based understanding of drug resistance, particularly those mediated by the TME, by accurately replicating the complex TME and its associated gradients [100].

Compared to 2D models, 3D cell cultures, particularly those supported by biomaterials, provide a more precise prediction of drug effects and toxicity, thereby bridging the divide between preclinical models and human physiology [22]. By emulating the ECM, hydrogel matrices facilitate the formation of multicellular tumour spheroids (MCTSs) [100]. The architectural structure of these MCTS is highly valuable for the study of drug permeation, efficacy, and discharge, as it provides significantly greater resistance to antineoplastic agents, similar to solid tumours in vivo [38]. This implies that biomaterials create a realistic TME that enables researchers to investigate the reasons why medications fail in vivo, such as diffusion barriers, hypoxic core, and ECM interactions, rather than merely determining whether they fail. As a critical stage in the development of more effective cancer therapies, this shifts drug discovery towards mechanism-based approaches for overcoming resistance.

The gene expression profiles of 3D MCTS, such as survival, proliferation, and resistance genes, are more similar to in vivo tumours than those observed in 2D cultures, as revealed by chemo-sensitivity analyses [101]. The role of the ECM provided by biomaterials in 3D systems in mediating chemo-resistance is significant, as it mirrors the physiological ECM's influence during chemotherapy [95]. To further improve the predictive potential and physiological relevance of drug screening platforms, biomaterials can be engineered to include features such as biochemical gradients and vascularization. This facilitates a more thorough comprehension of the TME's role in drug resistance, extending beyond basic cytotoxicity evaluations to investigate intricate molecular and cellular mechanisms.

Synergistic integration: omics technologies, computational models, and biomaterials

The integration of biomaterials with computational models and omics technologies is on the brink of revolutionising toxicology assessment through a synergistic process. This convergence facilitates the rational design of biomaterials, accelerates the discovery of safer and more effective therapies, and enables a more comprehensive understanding of molecular mechanisms of toxicity. The integration framework combining biomaterials, omics platforms, and computational models for predictive toxicology is illustrated in Figure 5.

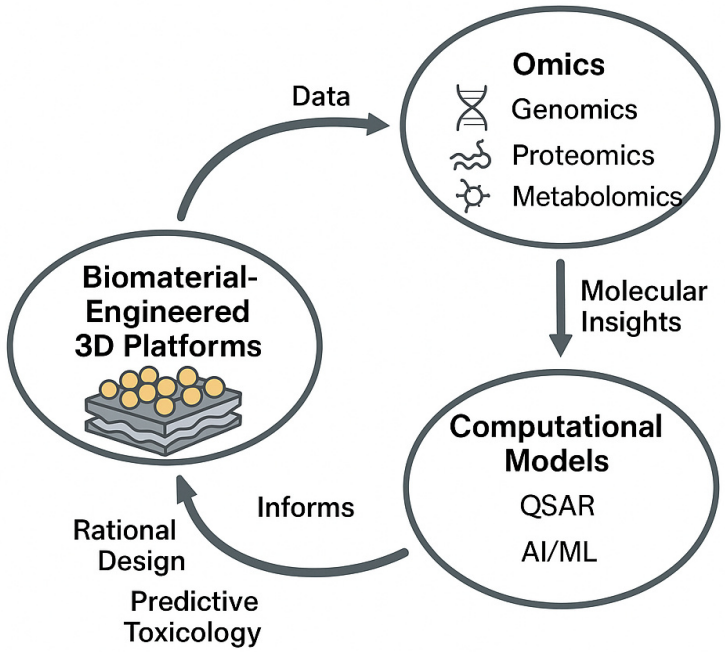


Figure 5. Synergistic integration: biomaterials, omics, and computational approaches. A flow diagram depicting the synergistic integration of biomaterial-engineered 3D platforms with advanced omics technologies (genomics, proteomics, metabolomics) and computational models (QSAR, AI/ML). This integration creates a powerful feedback loop where data from biomaterial models informs molecular insights via omics, which then fuels computational predictions for rational design in predictive toxicology and personalized medicine. Created using Napkin AI.

Leveraging biomaterial platforms to produce high-throughput omics data

By integrating genomics, transcriptomics, proteomics, and metabolomics, omics technologies offer an unparalleled opportunity to develop a thorough comprehension of the molecular mechanisms of toxicity. By capturing global changes in gene expression, protein abundance, and metabolite levels, these technologies can identify potential biomarkers of exposure or effect and enable personalised risk assessment [102].

Biomaterial-engineered 3D cell cultures are becoming more amenable to HTS, which enables the creation of large-scale omics datasets that are abundant in biological information [103]. The burgeoning discipline of "materiomics," which employs omics methodologies to analyse biomaterials, has the potential to expedite the development of medical devices by elucidating the relationships between material properties and their impact on intricate biological systems [104]. The analysis of biological effects induced by material properties is significantly improved by computational modelling, which is essential for the correlation of high-throughput gene expression profiling with combinatorial material design strategies. This integration enables the construction of libraries with high-throughput data on both biological (transcriptome) and material (materiome) scales, thereby enabling potent correlation analyses to define the biological functionality of material properties and enhance material design.

Application of computational models (QSAR, AI/ML) for rational biomaterial design and predictive toxicology

Computational models, including Artificial Intelligence/Machine Learning (AI/ML) algorithms [105] and Quantitative Structure-Activity Relationship (QSAR) models [105], offer cost-effective and time-efficient alternatives to traditional animal testing for assessing chemical hazards. These models predict chemical activity, carcinogenicity, immunotoxicity, and ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties by utilizing structural features and comprehensive datasets. AI/ML algorithms can discern patterns, correlations, and predictive relationships that remain obscured through conventional statistical methods by analyzing extensive datasets from sources like ToxCast, ChEMBL, and PubChem [106].

The design and optimization of novel biomaterials for in vitro models are increasingly informed by computational models, especially AI/ML and QSAR, which are advancing beyond simple toxicity prediction. This has led to the creation of a robust iterative feedback cycle that enhances the speed of material discovery and refinement. This represents a significant shift from the traditional trial-and-error methodology in biomaterial development. Computational models enable in silico parameter optimization for the rational design and development of novel biomaterials, thus streamlining the development process by allowing the variation of parameters that are difficult to modify in a laboratory setting. QSAR models are utilized to predict essential parameters for in vitro to in vivo extrapolation (IVIVE), thus connecting in vitro results with systemic effects [107]. These models are employed to enhance the physical, chemical, and biological characteristics of biomaterials for targeted in vitro applications. This process serves as the fundamental mechanism for rational design.

Challenges and opportunities in integrating multi-omics data with biomaterial-based in vitro models

Despite significant potential, considerable challenges exist in integrating multi-omics data with biomaterial-based in vitro models. The challenges encompass the inherent complexity of data analysis and interpretation, the necessity of ensuring data quality and completeness, and the difficulties posed by the "black box" nature of certain AI/ML models, which complicates mechanistic understanding [108]. The properties of polymers are significantly affected by molecular weight, distribution, and processing, resulting in the absence of straightforward, universal correlations between biological activity and chemical structure.

This integration presents numerous opportunities. The comprehensive biological cascade of effects is elucidated through the synergistic integration of multi-omics data, offering a more systemic and holistic understanding of toxicity pathways, thereby exceeding the limitations of single-endpoint analyses [104]. The integration of individual variability in genomic, biological, and physiological parameters facilitates personalized risk assessment. This process accelerates the identification of biomarkers by detecting molecular signatures linked to toxicity and chemical exposure. The integration of advanced in vitro models allows AI/ML to enhance the predictability of human responses by considering complex factors, including environmental influences, lifestyle variations, genetic diversity, and drug metabolism in preclinical studies. The field of predictive toxicology is poised for a significant transformation due to the integration of biomaterial-based in vitro models with multi-omics data, providing a more comprehensive, personalized, and systemic understanding of chemical-biological interactions. This method goes beyond simple correlations to clarify complex biological networks and pathways, offering a more thorough basis for drug development and safety evaluation.

Conclusion and future perspectives

This review has highlighted the essential function of biomaterials in the transition from conventional, frequently unreliable, toxicology models. Biomaterials provide the foundation for advanced 3D in vitro systems, such as spheroids, organoids, and OoC platforms, by engineering microenvironments that accurately replicate the compositional, structural, and mechanical cues of the native human ECM. These

models demonstrate enhanced physiological relevance relative to their 2D and animal counterparts, thereby offering a more precise framework for evaluating human-specific immunotoxicity and carcinogenicity. This advancement directly addresses the notable translational gaps, ethical issues, and financial challenges associated with traditional methods.

The potential of these platforms is enhanced by their integration with omics technologies and computational methods. This convergence establishes a robust feedback loop: biomaterial-engineered models produce high-quality, human-relevant omics data, which subsequently enhances predictive computational models such as AI/ML and QSAR. This synergy offers enhanced molecular understanding of toxicity mechanisms and facilitates the systematic design of safer therapeutics and advanced biomaterials, thereby expediting the drug discovery process.

Despite the significant potential, various challenges need to be resolved for broad adoption and regulatory approval. A significant challenge is the absence of standardization in the fabrication and maintenance of complex 3D cultures, resulting in variability that obstructs inter-laboratory comparisons. A trade-off exists between physiological complexity and high-throughput capability, resulting in sophisticated models that are costly and challenging to scale for mass screening. Moreover, accurately representing the dynamic complexity of the human immune system presents a considerable challenge, as many existing models do not incorporate a comprehensive, integrated immune component essential for effectively testing novel immunotherapies.

The integration of material science, advanced biology, and computational power is expected to transform toxicology and personalized medicine significantly. The field is advancing towards the development of patient-derived "avatars," including organoids and bioprinted tissues constructed from a patient's own cells, to predict individual drug responses with remarkable precision. This advancement establishes a definitive trajectory toward achieving the 3Rs objective: A future characterized by animal-free safety assessments in which more predictive, human-relevant NAMs are standardized. The ongoing advancement of "smart" biomaterials—capable of dynamically responding to stimuli or actively monitoring cellular health—will enhance these models from passive scaffolds to active, information-rich systems, offering real-time insights into biological processes. The application of these innovations will result in safer and more effective therapies, ushering in a new era of predictive and personalized healthcare.

Abbreviations

2D: two-dimensional

3D: three-dimensional

AI/ML: Artificial Intelligence/Machine Learning

ECM: extracellular matrix

EMT: epithelial-to-mesenchymal transition

FRCs: fibroblastic reticular cells

HTS: high-throughput screening

MCTSs: multicellular tumour spheroids

MPS: microphysiological systems

NAMs: New Approach Methodologies

OoC: Organ-on-Chip

PBMCs: peripheral blood mononuclear cells

PDTOs: patient-derived tumour organoids

QSAR: Quantitative Structure-Activity Relationship

TDAR: T-cell dependent antibody responses

TME: tumor microenvironments

Declarations

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Author contributions

SC: Conceptualization, Writing—original draft, Visualization. AD: Methodology, Writing—original draft, Visualization. AS: Methodology, Writing—original draft. PZ: Writing—review & editing. NG: Writing—review & editing. RS: Writing—review & editing. VT: Writing—review & editing. LMA: Writing—review & editing, Supervision. DG: Conceptualization, Writing—review & editing, Supervision. All authors have read and approved the submitted version.

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