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REVIEW

Modeling the Melanoma Tumor Microenvironment: From 3D Cultures to Predictive 4D *In Vitro* Platforms

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ABSTRACT: Melanoma is an aggressive skin cancer characterized by marked cellular heterogeneity and complex, dynamic interactions with the surrounding tumor microenvironment, which comprises fibroblasts, immune and endothelial cells, and the extracellular matrix. These interactions critically influence tumor progression, invasion, immune evasion, and the frequent emergence of resistance to targeted therapies and immunotherapies, underscoring the need for preclinical models that faithfully reproduce the complexity of human melanoma. Conventional two-dimensional (2D) culture systems, although still widely used, fail to recapitulate the structural architecture, cellular heterogeneity, and dynamic biochemical and mechanical cues of the tumor microenvironment, which limits their predictive value for therapeutic responses. Advanced three-dimensional (3D) and four-dimensional (4D) *in vitro* platforms have therefore emerged as more physiologically relevant alternatives capable of reproducing tissue-like organization and temporal tumor dynamics. The aim of this narrative review is to provide a critical overview of these advanced melanoma modeling platforms, including scaffold-free and scaffold-based 3D systems, spheroids, patient-derived organoids, assembloids, bioprinted constructs, and microfluidic tumor-on-chip devices, together with 4D time-resolved approaches. The review further examines the integration of high-resolution imaging, spatial omics, computational modeling, and artificial intelligence (AI)-based analysis as complementary tools for the quantitative, spatial, and temporal characterization of melanoma. By outlining the biological rationale and the technological landscape underlying these systems, this work aims to frame their potential contribution to more predictive, physiologically relevant, and translational melanoma research.

KEYWORDS: Melanoma; 3D culture; tumor-on-chip; 4D models; artificial intelligence; tumor microenvironment (TME)

1 Introduction

Melanoma arises from the malignant transformation of melanocytes and is highly influenced by interactions with the surrounding microenvironment, which includes fibroblasts, immune cells, endothelial cells, and the extracellular matrix (ECM) [1]. Fibroblasts, in particular, play a pivotal role by remodeling the tumor microenvironment (TME) and creating pre-metastatic niches that facilitate invasion and metastasis [1]. Dysregulation of key signaling pathways, including Mitogen-Activated Protein Kinase (MAPK) and p90RSK/MDM2/p53, drives proliferation, survival, and resistance to targeted therapies [2]. Epigenetic regulation through long noncoding RNAs and microRNAs further contributes to melanoma progression and treatment response [3,4].

The frequent emergence of resistance within six to eight months after treatment highlights the urgent need for preclinical models that accurately reflect the complexity of human melanoma [5]. In addition, the invasive and metastatic behavior of melanoma is driven by molecular mechanisms that regulate tumor cell migration and interaction with the surrounding microenvironment, further emphasizing the need for physiologically relevant experimental models [6].

While 2D culture systems are still widely used, they fail to replicate the structural complexity, cellular heterogeneity, and dynamic interactions of the TME [7]. In contrast, 3D culture systems allow direct cell-cell contact, ECM organization, and physiologically relevant mechanical and biochemical cues, providing a more faithful *in vitro* approximation of *in vivo* conditions [8]. The composition of the TME—including the relative abundance of cancer-associated fibroblasts, endothelial cells, immune populations, and ECM components—has been increasingly recognized as a key determinant of tumor behavior, therapeutic response, and prognosis, not only in melanoma but across multiple solid tumor types [1,8]. Spatial omics approaches have recently provided high-resolution maps of these compositional features within tumor tissues, revealing the prognostic and functional relevance of distinct microenvironmental niches. The presence or absence of key TME components in advanced *in vitro* platforms is therefore a critical factor influencing their predictive value and translational applicability. The aim of this review is to provide an overview of current 3D and 4D melanoma models, highlighting their applications in tumor microenvironment reconstruction, preclinical drug testing, and precision medicine.

Literature Search Strategy

This work was designed as a narrative review. The literature was identified through searches of PubMed, Scopus, and Web of Science using combinations of the following keywords: 'melanoma', '3D culture', 'spheroids', 'organoids', 'assembloids', 'bioprinting', 'tumor-on-chip', 'microfluidics', '4D tumor models', 'spatial omics', and 'artificial intelligence'. Original articles, reviews, and methodological studies published in English were considered. Studies not related to melanoma or advanced *in vitro* tumor models were excluded. As this work was designed as a narrative review, a formal PRISMA-guided systematic review process and meta-analysis were not performed. Formal risk-of-bias assessment and evidence-grading methodologies (e.g., GRADE) were not applied; this limitation is acknowledged in the Discussion.

2 Scaffold-Free and Scaffold-Based 3D Models

3D Platforms can be broadly divided into scaffold-free and scaffold-based systems (Fig. 1).

Scaffold-free approaches rely on the spontaneous self-assembly of cells into multicellular spheroids using methods such as hanging drops, low-adhesion plates, or dynamic culture systems like spinner flasks and bioreactors. These spheroids establish physiologically relevant gradients of oxygen, nutrients, and waste, as well as heterogeneous microenvironments that resemble aspects of solid tumors *in vivo*, making them valuable for studying drug responses and screening anticancer compounds [9].

Scaffold-based systems address these limitations by embedding cells within natural or synthetic biomaterials that better mimic ECM properties *in vivo*. Natural polymers such as collagen, fibrin, gelatin, and other ECM-derived hydrogels provide structural support and biochemical cues that promote physiologically relevant cell-matrix interactions, multicellular organization, and signaling, supporting studies of cell invasion, migration, and therapy resistance that are difficult to capture with 2D cultures [10]. These natural scaffolds retain biologically active ECM components and cell adhesion motifs that actively influence cell behavior. As a result, cancer cells can adhere, proliferate, migrate, and interact with their surroundings

within a more physiologically relevant microenvironment, responding to mechanical and biochemical signals in a manner that more closely resembles *in vivo* tissues [10].

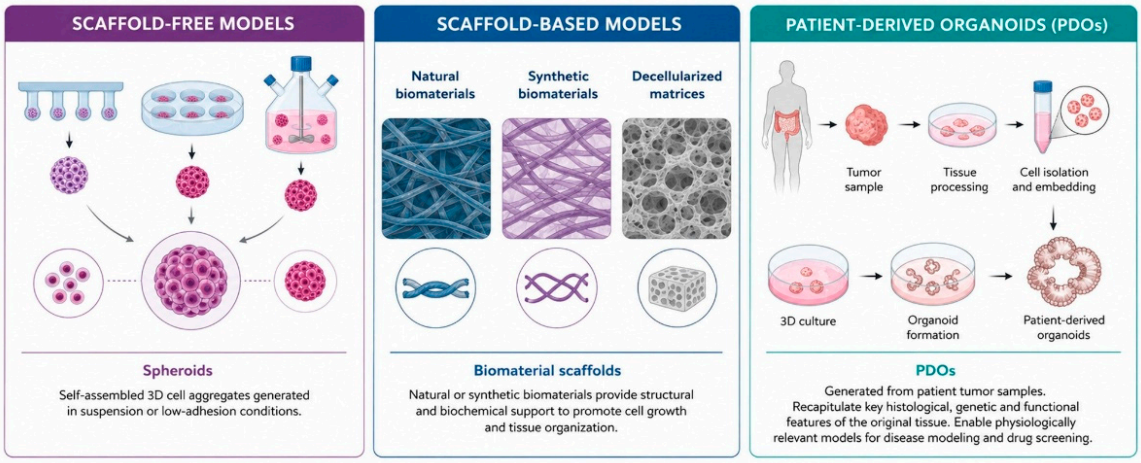

Figure 1: Overview of 3D *in vitro* tumor models. Main categories of 3D culture systems used in cancer research. Scaffold-free models include self-assembled spheroids, whereas scaffold-based systems use natural or synthetic biomaterials to support cell growth, ECM organization, and tissue architecture. Patient-derived organoids (PDOs), generated from tumor samples, preserve key histological, molecular, and functional features of the original tissue and represent physiologically relevant platforms for disease modeling, drug screening, and personalized therapeutic studies. The schematic illustration was assembled by the authors using Microsoft PowerPoint and subsequently refined for visual consistency with the assistance of ChatGPT (OpenAI), based on author-provided scientific instructions.

Decellularized extracellular matrix (dECM)-based scaffolds have emerged as particularly promising biomaterials for melanoma modeling because they preserve the biochemical composition, structural organization, and tissue-specific signaling properties of native extracellular matrices (Fig. 2) [11].

By maintaining collagen networks, glycoproteins, growth factors, and cell-adhesion motifs, dECM scaffolds provide highly biomimetic microenvironments that support tumor cell proliferation, invasion, and tumor-stroma interactions under physiologically relevant conditions.

Collagen I provides structural support and regulates melanoma cell adhesion and invasion, whereas fibronectin promotes cell migration through integrin-mediated signaling. Laminin contributes to cell adhesion, polarity, and invasive behavior, while glycosaminoglycans (GAGs) regulate growth factor availability and cell signaling within the tumor microenvironment. Elastin contributes to the biomechanical properties of the extracellular matrix, influencing tissue remodeling and melanoma cell migration. Together, these extracellular matrix components provide biochemical and mechanical cues that modulate proliferation, invasion, tumor–stroma interactions, and therapeutic response [1,11,12].

In addition, dECM-derived bioinks have been increasingly incorporated into bioprinted melanoma constructs to improve biological fidelity and reproduce tissue-specific mechanical and biochemical properties that are difficult to achieve using synthetic materials alone [12].

In addition to natural materials, synthetic polymers have been widely explored to create scaffold matrices with controllable mechanical and chemical properties that support 3D cell culture and tumor modeling. Polymers such as polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), and related derivatives can be tuned to provide specific stiffness, porosity, and degradation rates that influence cell proliferation, migration, and drug responses in engineered tumor environments [10].

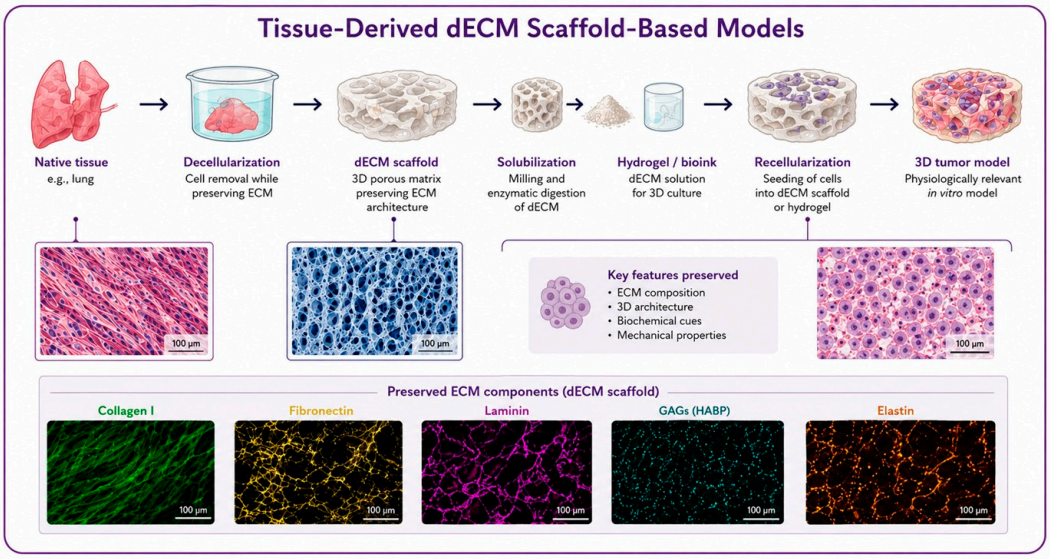

Figure 2: Tissue-derived decellularized extracellular matrix (dECM) scaffold-based melanoma models. Native tissues undergo decellularization to generate biomimetic ECM scaffolds that preserve the structural and biochemical properties of the original tissue. The resulting dECM can be processed into hydrogels or bioinks and subsequently recellularized with tumor cells to generate physiologically relevant 3D melanoma models. Representative histological and fluorescence images illustrate ECM preservation and the maintenance of key ECM components, including collagen I, fibronectin, laminin, glycosaminoglycans (GAGs), and elastin. The schematic illustration was assembled by the authors using Microsoft PowerPoint and subsequently refined for visual consistency with the assistance of ChatGPT (OpenAI), based on author-provided scientific instructions.

These scaffold materials, whether natural or synthetic, can be optimized to reproduce critical aspects of the tumor ECM, including structural support, mechanical stiffness, and biochemical signaling. Embedding cells within such defined matrices allows investigation of processes such as ECM remodeling, collective invasion, and therapy resistance under conditions that more closely resemble the native TME than traditional 2D cultures [13].

PDOs cultured within ECM matrices preserve inter and intratumoral heterogeneity, stromal components, and immune populations. These models have been successfully used to study anti-PD1 immunotherapy responses, Tumor-Infiltrating Lymphocytes (TIL) behavior, and small molecule inhibitors while maintaining immune contexture similar to the original tumor [14,15].

However, the broader implementation of PDOs is still limited by variable establishment and expansion rates, prolonged culture times, and the lack of standardized culture protocols, which may affect reproducibility and cross-laboratory comparability [14,15].

3 Advanced 3D Platforms: Assembloids, Bioprinting, and Microfluidics

Assembloids

Melanoma assembloids are modular 3D systems in which melanoma cells self-organize with stromal and immune populations, better capturing tumor heterogeneity, ECM deposition, and physiological nutrient and oxygen gradients. These models preserve rare subpopulations and support investigation of phenotype switching, stromal-mediated invasion, and therapy resistance over extended culture periods [16]. More broadly, advanced multicellular 3D platforms are increasingly being exploited to investigate complex inter-

actions within the tumor microenvironment, including tumor–microbiome crosstalk, thereby expanding their translational applications beyond conventional tumor modeling [17].

Bioprinting

Bioprinted constructs complement assembloids by providing precise spatial organization of melanoma cells, stromal components, and ECM, enabling recreation of more native-like tissue architecture with enhanced control over cellular composition and microenvironmental signals. These models allow detailed studies of invasion, ECM remodeling, and drug penetration, and they can incorporate multiple cell types in defined patterns that better mimic complex human tissues *in vitro* [18].

Advanced 3D bioprinted tumor constructs have also been used as predictive platforms for preclinical evaluation of therapeutic strategies, including combinatorial drug delivery approaches and immunotherapy resistance mechanisms, by enabling more accurate assessment of tumor-microenvironment interactions and treatment responses than conventional models [19].

Furthermore, high-throughput 3D bioprinted skin cancer organoids have been developed that integrate patient-derived cells and engineered extracellular matrices, and these platforms are discussed as promising tools for diagnostic screening and personalized medicine, bridging engineering innovations with translational research to accelerate therapeutic development and individual treatment strategies [20].

Microfluidic Platforms

Microfluidic melanoma-on-chip models provide precise control over chemical gradients, fluid flow, and mechanical forces, enabling detailed studies of tumor-stromal-immune interactions. By integrating endothelial, stromal, and immune compartments, these platforms replicate aspects of vascularization, nutrient delivery, and ECM remodeling that are absent in static 3D cultures. This allows researchers to investigate how fluid shear, oxygen gradients, and perfusion influence invasion, angiogenesis, and drug penetration, creating a physiologically relevant alternative to traditional spheroid or organoid systems [21–25].

However, fabrication complexity, device-to-device variability, and limited compatibility with routine high-throughput workflows remain important barriers to the broader adoption and standardization of microfluidic melanoma models [21–23].

Moreover, vascularized tumor-on-chip models facilitate the study of therapy resistance under dynamic conditions, including responses to targeted inhibitors and immunotherapies, while preserving cell heterogeneity and tumor-stroma interactions [21–23]. These systems also allow real-time monitoring of cellular behavior and detailed mechanistic investigation of tumor progression, including cancer cell invasion, phenotype switching, and interactions with stromal and immune components of the TME. In addition, the dynamic nature of microfluidic platforms makes it possible to study how biochemical gradients, interstitial flow, and mechanical forces influence melanoma progression and treatment response over time. By reproducing key physiological features that are difficult to achieve in conventional 2D cultures or static 3D models, these systems provide more reliable and predictive evaluations of drug efficacy, therapeutic resistance, and tumor-microenvironment interactions. The analytical and temporal dimensions of these platforms are further explored in Sections 4 and 5 through high-resolution imaging, spatial omics, AI-assisted analysis, and time-resolved 4D modeling.

Overall, microfluidic melanoma-on-chip platforms represent a major advancement in preclinical cancer modeling. The integration of controlled perfusion, spatial tissue organization, multicellular complexity, and physiologically relevant mechanical stimuli allows the generation of more biomimetic tumor models, improving the translational relevance of mechanistic studies and preclinical therapeutic testing [21–23,25].

4 Imaging, Spatial Omics, and AI Integration

Imaging

The utility of these platforms is further enhanced by high-resolution imaging, spatial omics, and AI-based analysis. Multiphoton and confocal microscopy allow real-time visualization of invasion fronts, vascular-like structures, and immune infiltration while preserving tissue architecture [21,26].

Spatial Omics

Spatial transcriptomics, proteomics, and metabolomics allow the analysis of gene expression, protein distribution, and metabolic activity while preserving the spatial organization of the tissue or engineered construct. These approaches make it possible to identify cellular heterogeneity, localized signaling pathways, and biochemical gradients within complex 3D models, revealing how different cell populations interact with each other and with the surrounding microenvironment. By maintaining spatial information, these techniques provide a more accurate understanding of tumor architecture, functional organization, and regional differences in treatment response [27].

Artificial Intelligence

AI-driven image analysis supports automated segmentation and quantitative assessment of tumor invasion, immune interactions, and therapy response, facilitating high-content and reproducible analysis of advanced *in vitro* models [26].

Current AI applications in advanced melanoma models include convolutional neural networks (CNNs) for image segmentation and machine-learning approaches for drug-response prediction [28,29]. More broadly, recent advances in artificial intelligence have introduced transformer-based architectures, multimodal analytical frameworks, and computer vision approaches that further expand the potential applications of AI in melanoma research [30,31]. However, many published studies rely on relatively small datasets, and external validation across independent cohorts remains limited. Challenges related to model generalizability, dataset standardization, explainability, and clinical implementation continue to represent major barriers to translation [28–31]. When integrated with longitudinal imaging and time-resolved experimental platforms, these AI-based approaches can facilitate the quantitative analysis and interpretation of dynamic tumor-immune interactions and treatment responses over time.

Beyond descriptive image analysis, AI is progressively evolving toward predictive modeling. By integrating high-content imaging with spatial omics and molecular datasets, machine-learning frameworks can support the prediction of drug response, therapy resistance, and tumor evolution, thereby enhancing the translational value of advanced melanoma models. However, standardized quantitative benchmarks, external validation across independent datasets, and harmonized performance metrics remain limited. Establishing robust validation frameworks will therefore be essential before these approaches can be routinely implemented in advanced 3D and 4D melanoma platforms [28–31].

Limitations of AI-Based Imaging in Pigmented Melanoma Models

An important melanoma-specific limitation of fluorescence-based imaging approaches must be acknowledged in this context. The strong endogenous pigmentation characteristic of many melanoma models can generate optical artifacts that reduce the quality and reliability of confocal microscopy and live-cell tracking, particularly in deeply pigmented 3D and 4D constructs where melanin absorption and scattering interfere with signal detection. AI-based image analysis pipelines face specific challenges under these conditions, including the risk of misclassification arising from melanin-related autofluorescence, which can be mistaken for fluorescent reporter signals. Dedicated validation of AI analytical tools on pigmented melanoma models remains limited and represents a gap that future methodological work should address [32].

5 4D Models and Computational Approaches

The term "4D" refers to three-dimensional culture systems that incorporate a fourth dimension: time. The defining feature of 4D approaches is thus time-resolved, longitudinal observation, not merely static three-dimensional organization. While 3D models capture spatial complexity, including tumor architecture, ECM organization, and multicellular interactions, 4D systems additionally enable continuous monitoring of how these structures and behaviors evolve over time. In this framework, 3D platforms provide a spatially accurate snapshot of the TME, whereas 4D platforms extend this by tracking dynamic processes, such as proliferation, phenotype switching, invasion, and therapy-induced adaptation within living tumor constructs over extended periods. Specific tools enabling the 4D dimension include the Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI) system for real-time cell-cycle tracking, stochastic and mathematical modeling for simulating temporal tumor dynamics, and AI-based image analysis for automated longitudinal quantification. Together, these approaches define the 4D concept and distinguish it from conventional 3D culture.

Unlike conventional 3D models, 4D platforms incorporate the temporal dimension, allowing continuous monitoring of how tumor architecture, cellular behavior, and treatment responses evolve over time. These systems support longitudinal analysis of dynamic processes such as proliferation, phenotype switching, invasion, and therapy-induced adaptation within living tumor constructs. One commonly used approach is the FUCCI system, which labels cells in different phases of the cell cycle with distinct fluorescent signals, enabling real-time tracking of cell cycle progression and proliferative dynamics in living spheroids or organoids [33]. By monitoring temporal changes in proliferation, necrosis, and therapy response, 4D models make it possible to study tumor evolution and adaptive cellular behaviors over time, providing biologically relevant information that static 3D cultures cannot accurately capture.

These dynamic platforms are often combined with stochastic and mathematical modeling, which simulate tumor growth, nutrient gradients, cell cycle progression, and the effects of drugs. By combining experimental and computational approaches, these systems help predict tumor behavior, disease evolution, and therapeutic outcomes under different physiological and treatment conditions [34,35]. For example, computational modeling of 4D tumor spheroids has shown that differences in cellular proliferation rates within distinct regions of the spheroid can promote therapy resistance and enhance invasive behavior, highlighting the impact of intratumoral heterogeneity on tumor progression and treatment response.

AI-based image analysis further enhances these platforms by enabling automated, high-resolution quantification of dynamic cellular processes within complex tumor models. In melanoma-on-chip systems, deep learning algorithms can continuously monitor immune cell infiltration, tumor cell migration, and temporal changes in tissue architecture, generating objective and quantitative measurements of tumor progression, immune interactions, and therapeutic response. This approach improves the accuracy, reproducibility, and throughput of data analysis while reducing observer-dependent variability [28,29]. By integrating FUCCI-based imaging, computational modeling, and AI-driven analytical approaches, researchers can investigate not only the spatial organization and heterogeneity of tumors but also their dynamic temporal evolution. This integrated strategy allows continuous monitoring of proliferation, invasion, cellular adaptation, and treatment response over time, ultimately improving the physiological relevance and predictive accuracy of preclinical tumor models.

Overall, advanced 3D and 4D melanoma platforms, including assembloids, bioprinted constructs, microfluidic chips, and PDOs, provide unprecedented opportunities to investigate tumor biology, therapeutic responses, and immune interactions under physiologically relevant conditions that closely recapitulate the human TME. By bridging the gap between conventional *in vitro* models and clinical reality, these systems support mechanistic studies, high-throughput drug screening, and the development of personalized therapeutic

strategies. In addition, their dynamic and multicellular nature allows real-time monitoring of tumor-immune interactions, cellular adaptation, and treatment induced changes over time [16,18,28]. The integration of temporal monitoring, computational simulations, and AI-assisted quantitative analysis transforms melanoma modeling into a predictive, patient-specific, and physiologically relevant platform, providing deeper insights into treatment efficacy, resistance mechanisms, and tumor-immune dynamics [28,29,34]. The principal features and comparative advantages of the melanoma *in vitro* models discussed throughout this review are summarized in Table 1.

Table 1: Comparison of conventional, 3D, and advanced melanoma *in vitro* models. Overview of the structural complexity, tumor microenvironment (TME) representation, cell-cell and cell-matrix interactions, dynamic properties, immune relevance, and predictive potential of conventional 2D cultures and advanced melanoma platforms, including spheroids, scaffold-based systems, PDOs, assembloids, bioprinted constructs, microfluidic systems, and 4D models. Qualitative ratings, including Low, Moderate, High, and Very high, reflect the relative capacity of each platform to reproduce the indicated biological feature based on current literature. These ratings are intended as comparative indicators rather than absolute quantitative measures. The qualitative ratings were assigned by integrating evidence from the cited literature according to each platform's reported ability to reproduce tumor architecture, extracellular matrix composition, multicellular interactions, immune components, dynamic behavior, and predictive performance in preclinical applications [7–25].

Model	Structural Complexity	TME Representation	Cell-Cell/Cell-Matrix Interactions	Dynamics (Time Dimension)	Immune Interactions	Predictive Potential
2D cultures	Flat monolayer	None	Absent	Absent	Absent	Low
Spheroids	3D aggregates	Tumor cells ± fibroblasts	Moderate to high (mainly cell-cell interactions)	Limited	Absent	Moderate
Scaffold-based models	3D + ECM	Tumor cells + ECM ± fibroblasts ± endothelial cells	Moderate (enhanced cell-matrix interactions)	Limited	Absent	Moderate
Organoids (PDOs)	Complex 3D	Tumor epithelial cells ± stromal and immune components	High (physiological cell-cell and ECM interactions)	Limited	Partial (if immune components are retained or added)	High
Assembloids	Multicellular	Tumor cells + fibroblasts + endothelial ± immune cells	High (multicellular interactions across compartment)	Moderate	High (multicellular immune interaction)	High
Bioprinted models	Controlled architecture	Tumor cells + fibroblasts + endothelial ± immune cells	High (spatially controlled cell-cell and ECM interactions)	Moderate	Moderate (engineered inclusion of immune cells)	High
Microfluidic systems	Perfused, multicompartment	Tumor cells+ fibroblasts + endothelial + immune cells	High (dynamic cell-cell and cell-matrix interactions under flow)	High (dynamic perfusion and controlled flow)	High (dynamic immune interactions under flow)	Very high
4D models	Complex dynamic 3D architecture	Tumor cells + full TME (stromal + immune)	High (time-evolving and adaptive interactions)	Very high (time-resolved, dynamically evolving systems)	Very high (time-resolved immune responses)	Very high/patient-specific potential

Note: 2D, two-dimensional; 3D, three-dimensional; 4D, four-dimensional; ECM, extracellular matrix; PDOs, patient-derived organoids; TME, tumor microenvironment.

6 Discussion

Next-generation 3D and 4D melanoma platforms, including spheroids, organoids, assembloids, bioprinted constructs, microfluidic chips, represent a substantial advancement over conventional 2D culture systems [16,18,36]. By incorporating tumor architecture, stromal support, immune components, ECM organization, and microenvironmental signaling, these models more closely reproduce key features of the human melanoma microenvironment. In particular, self-organizing assembloids capture heterotypic cell interactions and tumor-stromal dynamics with greater biological fidelity than simple spheroids, providing valuable insight into melanoma progression, invasion, and microenvironment-driven plasticity [16,17]. Similarly, advanced bioprinted melanoma models allow precise spatial organization of multiple cell types and ECM components, supporting controlled investigation of tumor behavior, matrix remodeling, and therapeutic response in more physiologically relevant settings [18–20].

Compared with traditional *in vitro* systems, these platforms provide more reliable experimental conditions for studying melanoma heterogeneity, invasion, metastasis, immune interactions, and drug resistance. The incorporation of stromal, vascular, and immune elements strengthens their translational relevance by recreating critical aspects of the TME that influence therapeutic efficacy and resistance mechanisms [18–20].

Compared with conventional spheroids, PDOs generally preserve a higher degree of inter and intratumoral heterogeneity and more closely recapitulate the molecular characteristics of the original tumor [14,15]. Microfluidic systems provide superior control over perfusion, nutrient gradients, and dynamic cell-cell interactions, supporting more physiologically relevant studies of tumor progression and therapeutic response [21–25]. Bioprinted constructs offer enhanced spatial reproducibility and architectural control, facilitating the standardized incorporation of stromal and vascular components, although they are often associated with higher technical complexity and costs [18–20]. Conversely, spheroids remain the most accessible, cost-effective, and scalable platforms for high-throughput drug screening, making them particularly suitable for large-scale preliminary therapeutic evaluations [9,13,22]. Overall, no single platform currently reproduces all aspects of melanoma biology, and the choice of model should be guided by the specific biological question, balancing physiological relevance, experimental complexity, reproducibility, and throughput [13,22,23].

Beyond the selection of a single platform, growing evidence supports the complementary and integrated use of multiple melanoma models within a tiered experimental workflow that leverages the distinct strengths of each system while compensating for their individual limitations. In such a strategy, cost-effective and scalable scaffold-free spheroids are well suited to initial high-throughput drug screening and dose-finding; shortlisted candidates and mechanistic hypotheses can then be validated in patient-derived organoids and assembloids, which preserve inter- and intratumoral heterogeneity, stromal architecture, and immune contexture. Microfluidic tumor-on-chip and vascularized platforms subsequently allow the interrogation of perfusion-, shear-, and gradient-dependent processes such as angiogenesis, drug penetration, and immune trafficking under dynamic conditions, while 4D time-resolved systems add the longitudinal dimension required to capture phenotype switching, proliferation dynamics, and therapy-induced adaptation. Rather than competing alternatives, these platforms are therefore best regarded as complementary components of an integrated modeling pipeline, in which spatial omics and AI-assisted quantitative analysis provide a common analytical framework that links molecular composition, spatial organization, and temporal behaviour across model systems. Such cross-platform integration, together with harmonized quantitative readouts and standardized validation criteria, is expected to improve the reproducibility, comparability, and

translational predictive value of melanoma modeling beyond what any individual platform can achieve in isolation [13,16,18,22,23,27].

It should be noted that each platform presents distinct advantages and limitations. Importantly, no single model currently reproduces all aspects of melanoma biology, and model selection should therefore be guided by the specific biological question being addressed.

Although multiple studies suggest that organoids, bioprinted constructs, and microfluidic platforms may improve the prediction of therapeutic responses compared with conventional 2D cultures, direct quantitative comparisons remain challenging. Standardized predictive performance metrics, such as sensitivity, specificity, area under the curve (AUC), and drug-response correlation coefficients, are not consistently reported across studies, limiting objective assessment of the relative predictive value of different model systems.

When combined with 4D approaches, high-resolution imaging and spatial omics enable longitudinal assessment of tumor evolution, immune infiltration, and extracellular matrix remodeling [27,33]. Computational modeling and AI-assisted analysis further support the investigation of cell-cycle progression, oxygen and nutrient gradients, and treatment adaptation over time by providing quantitative and predictive analytical tools [28–35]. Together, these integrated strategies generate temporally and spatially resolved information that is difficult to obtain from static 3D cultures alone.

Translational Limitations

Important translational limitations nevertheless remain. Most *in vitro* systems are unable to fully reproduce systemic immune responses, endocrine regulation, lymphatic drainage, and the complex interactions occurring among distant organs during tumor progression and metastasis [21–25]. Maintaining long-term model stability while preserving cellular heterogeneity and functional characteristics also remains challenging, particularly for patient-derived organoids [14,15]. Another limitation is the incomplete integration of clinical, genomic, and multi-omics patient-derived data into experimental models, which may reduce their predictive value for personalized therapeutic responses [14,27]. Furthermore, multicenter validation studies are still limited, making it difficult to assess the reproducibility and generalizability of findings across different laboratories and experimental settings [10,11,22,23]. A further limitation of the present review is the substantial heterogeneity among the included studies in terms of experimental design, model type, culture conditions, endpoints, and reporting methodologies. Consequently, quantitative meta-analysis and pooled statistical evaluation were not feasible, and direct comparisons among studies should therefore be interpreted with caution.

Furthermore, because this work was designed as a narrative review, formal risk-of-bias assessment and evidence-grading methodologies were not applied. Future systematic reviews focusing on specific categories of melanoma models may benefit from structured quality assessment frameworks to facilitate objective comparison among studies.

Future Perspectives

Addressing these translational gaps will be essential to strengthen the clinical relevance of next-generation melanoma platforms and facilitate their broader implementation in precision oncology.

Patient-derived and immune-competent models are particularly promising for personalized oncology, as they preserve patient-specific tumor features and may support *ex vivo* evaluation of therapeutic responses and individualized treatment strategies [37]. Advanced bioprinted melanoma models incorporating multiple cell types provide physiologically relevant platforms for preclinical drug testing and therapeutic evaluation, highlighting their potential role in precision oncology and translational research [18,38].

Future efforts should prioritize the development of standardized culture protocols, harmonized quality-control criteria, integration of patient-derived multi-omics datasets, and multicenter validation studies.

Future studies should also focus on the development of benchmark datasets and standardized response metrics to enable direct comparison among melanoma modeling platforms and facilitate quantitative assessment of predictive performance across studies. In addition, the combination of immune-competent microfluidic platforms, advanced bioprinting technologies, and AI-assisted analytical approaches may further improve the predictive accuracy and clinical applicability of next-generation melanoma models. These advances may also contribute to reducing dependence on animal experimentation while providing deeper insight into the biological mechanisms underlying melanoma progression, treatment response, and resistance [10,18,27–29].

Further progress will require improving model reproducibility, reducing technical complexity, harmonizing validation criteria, and integrating standardized quantitative readouts. The combination of patient-derived models, refined microfluidic perfusion, advanced bioprinting, multi-omics profiling, computational simulations, and AI-driven predictive analysis may transform melanoma modeling into a more accurate and clinically informative preclinical framework.

Among the translational gaps requiring future attention, the optical interference caused by endogenous melanin pigmentation in 3D and 4D melanoma constructs remains an underappreciated technical challenge, particularly for fluorescence-based imaging and AI-driven analytical workflows.

Remaining Practical Challenges

Despite this progress, important limitations remain. Reproducibility and standardization across laboratories are still major challenges, especially for patient-derived systems, natural ECM scaffolds, and complex multicellular constructs. Batch-to-batch variability, elevated costs, technical expertise, prolonged culture times, and limited compatibility with high-throughput workflows may restrict broader implementation. In addition, immune-competent *in vitro* models provide detailed information on local tumor-immune interactions but still cannot fully reproduce systemic immunity, endocrine regulation, lymphatic drainage, or circulating factors present *in vivo* [18].

Practical challenges related to standardization, scalability, and cost represent substantial, unresolved barriers that must be explicitly acknowledged. While spheroids can be adapted to high-throughput screening formats with relative ease, organoids, assembloids, and microfluidic systems typically require specialized expertise, prolonged culture periods, and considerably higher costs, which may limit their large-scale implementation in routine preclinical workflows. PDOs may exhibit variable establishment and expansion rates that affect reproducibility and scalability across different tumor samples and laboratories [37]. Bioprinting requires highly optimized bioinks and specialized fabrication equipment that are not widely available, and the performance of printed constructs can be sensitive to minor variations in bioink composition and printing parameters [38]. Microfluidic platforms remain particularly difficult to standardize due to device-to-device variability, fabrication complexity, and limited compatibility with routine high-throughput workflows. Reproducibility is a major concern for patient-derived and dECM-based models, where biological variability in source materials introduces additional heterogeneity. The lack of standardized protocols for model generation, quality control, and functional validation represents a key barrier to broader adoption and cross-laboratory comparability. These are not minor caveats but substantial challenges that the field must prioritize in order to realize the translational potential of advanced melanoma platforms.

7 Conclusions

In conclusion, advanced 3D and 4D melanoma models are reshaping preclinical cancer research by combining biological fidelity with quantitative, temporal, and spatial analyses. Continued refinement of these platforms, together with progress in imaging, computational methods, and multi-omics integration, is

expected to improve therapeutic testing, support patient-specific treatment strategies, and strengthen the connection between experimental melanoma research and clinical application [16].

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