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REVIEW

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3D bioprinted melanoma models: a novel paradigm for the assessment of anticancer strategies combining PDT and drug delivery systems

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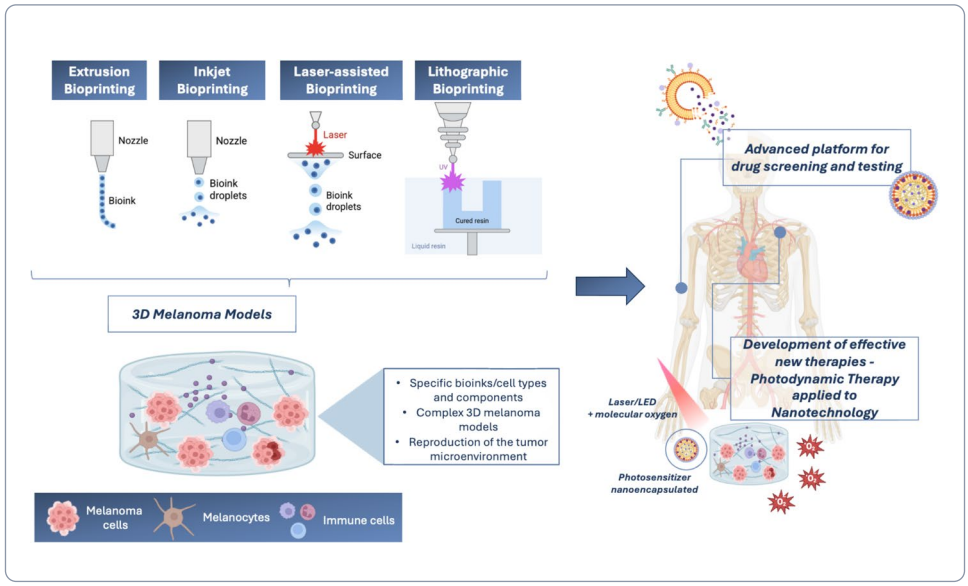
Abstract

Malignant melanoma remains the most aggressive type of skin cancer, leading to a high rate of associated death over the past decade, often exhibiting resistance to conventional therapies and presenting significant challenges for preclinical testing. In this instance, the complexity of the progression and the interaction within the tumor microenvironment highlight the necessity for advanced models. Traditional 2D cultures and standard 3D systems, such as spheroids and organoids, fail to fully replicate native skin architecture and lack reproducibility, vascularization, and immune integration. Recent advances in 3D bioprinting have enabled the development of melanoma models that more accurately mimic human skin by incorporating multiple cell types, extracellular matrix components, and spatial control. These models support the evaluation of innovative therapies, including nanocarrier-based drug delivery systems and photodynamic therapy (PDT). This review discusses the evolution of in vitro melanoma modeling, highlighting the role of bioprinting technologies and bioink design within this setting, and investigates emerging applications in PDT and drug delivery systems, assessing the advances and current challenges in the context of melanoma.

Keywords: Malignant melanoma, 3D bioprinted models, Tissue engineering, Drug delivery systems, Photodynamic therapy, 3D bioprinting

Graphical abstract



Introduction

Melanoma is considered to be one of the most aggressive types of skin cancer, originating from melanocytes located in the epidermis [1]. According to the International Agency for Research on Cancer (IARC), although it represents the smallest fraction in all skin cancer cases, melanoma is responsible for more than 60% of deaths in Europe and North America and for two-thirds of skin cancer deaths globally [2].

The progression of the disease results from a complex interaction between genetic and environmental factors, with exposure to ultraviolet (UV) radiation being the main risk factor, as it induces direct and indirect damage to melanocyte DNA [3]. Hereditary cases are often associated with mutations in genes such as CDKN2A, CDK4, and BAP1, although they represent a small proportion of the incidence [4]. In addition to molecular changes, melanoma is strongly influenced by its tumor microenvironment, where interactions between tumor cells, extracellular matrix (ECM), and stromal cells play a central role in progression, immune evasion, and therapeutic resistance [5].

Traditional in vitro monolayer models are widely used in assays to evaluate melanoma biology, such as cell migration assays, cell internalization, and determination of cytotoxicity in melanoma cells [6]. However, these models fail to replicate complex three-dimensional interactions, limiting their applicability for investigating events such as matrix remodeling, local immune response, and paracrine signaling [7, 8].

Three-dimensional models such as spheroids and organoids have emerged as relevant alternatives in preclinical trials, approximating the cellular structure of tumor tissue. Spheroids allow the investigation of properties such as invasion, cellular heterogeneity, and drug resistance, and organoids can follow co-culture protocols and use stem cells that mimic the physiological functions of the tissue [9, 10]. However, both models have limitations related to tissue architecture, lack of vascularization, and low reproducibility [11, 12].

As an emerging alternative, 3D bioprinting has been proposed as a promising approach for constructing more realistic tumor models in new anticancer therapies [13]. Through controlled deposition of cells and bioinks layer by layer, this technique allows for the

accurate replication of tissue spatial organization, incorporating tissue compartmentalization and vascularization [14]. In melanoma models, bioprinting has enabled the development of tumor tissue reconstructions, invasiveness assessment, and drug screening in a more predictive and personalized manner, when compared with traditional models (Table 1) [15, 16].

Given the advances in targeted therapies, the combination of photodynamic therapy (PDT) and drug delivery systems has shown promising results in the treatment of melanoma; especially due to its minimally invasive profile and ability to generate reactive oxygen species locally in tumor tissue [17]. In this context, 3D bioprinting emerges as an innovative approach in the development of more realistic models for melanoma studies, offering the possibility of more accurately investigating the therapeutic efficacy of formulations [18]. Thus, this review compiles and discusses recent advances in 3D bioprinting applied to the study of PDT for melanoma, addressing fundamental principles of this technology, challenges, and prospects in the development of more robust models and more effective therapeutic strategies.

Skin structure and melanoma biology

Skin anatomy and function

The human skin is composed of three main layers: epidermis, dermis, and hypodermis [19]. The epidermis is the outermost layer, protecting the skin from—among others—UV rays and pathogens through secreting antimicrobial proteins. This layer is primarily composed of keratinocytes (about 95%), with the remaining 5% consisting of melanocytes, Langerhans cells, and Merkel cells. Melanocytes are located in the epidermis and originate from neural crest-derived dendritic cells [20, 21]. Underneath the epidermis is the dermis, which contains interstitial components, such as collagen fibers, elastic tissue, and ground substance, presenting different cell types including fibroblasts, mast cells, plasma cells, lymphocytes, dermal dendritic cells, and histiocytes [22]. The dermis also stocks blood vessels, lymphatic channels, and sensory nerves [23]. It is divided into two regions: the papillary dermis, which interfaces with the basement membrane and is rich in capillaries and nerve endings; and the reticular dermis, which connects to the subcutis. The hypodermis, or subcutaneous layer, is composed mainly of adipose tissue and lies between the dermis and the underlying muscle. It provides mechanical protection and serves as a reservoir for mesenchymal stem cells [24].

Melanoma development and progression

Melanoma is a type of skin cancer originated from the transformation and uncontrolled proliferation of melanocytes, located in the basal layer—the stratum basale—of the epidermis. Cutaneous melanoma is classified in four primary subtypes, differing in their prognosis [25]. Superficial spreading melanoma is considered the most common type of melanoma (70%), presenting prolonged radial (horizontal) growth pattern on the skin before becoming invasive. It shows variation in color such as black, red, brown, blue, and white as pigment pattern and irregular asymmetric borders [26]. Nodular malignant melanoma represents 15% of the melanoma cases, presenting fast invasive profile after appearing. It shows dome-shaped dark brown or black nodule [27]. Acral lentiginous melanoma represents 8% of the melanoma cases.

Table 1 Comparison between 2D models, spheroids, organoids, and 3D bioprinted melanoma models for preclinical and therapeutic applications, assessing parameters such as cellular architecture, tumor microenvironment, clinical relevance, invasiveness and heterogeneity, vascularity, cost, reproducibility, and applications

Parameters	Monolayer [6–8]	Spheroids [9, 10]	Organoids [9, 10]	3D bioprinting models [14, 15]
Cellular architecture	Flat adherent cells, lacking spatial organization	Compact cellular aggregates with oxygen and nutrient gradients	Complex, self-organizing structure containing multiple cell types and tissue polarity	Personalized and controlled 3D tissue architecture
Tumor microenvironment	Minimal representation of ECM and stromal components	Preserves diffusion gradients and some cell–ECM interactions	Recapitulates tumor niches, paracrine signaling, and stromal–tumor interactions	Highly preserved with precise spatial control and accurately reproduced through bioinks
Clinical relevance	Limited predictive capacity and overestimation of drug sensitivity	Improved correlation with in vivo therapeutic response	High fidelity to the original tumor tissue and more predictable biological behavior	Advanced physiological modeling and therapeutic testing
Invasiveness and heterogeneity	Restricted to a bidimensional plane	Enable assessment of 3D invasion and drug resistance	Allows mapping of tumor heterogeneity and spatial organization	Allows accurate invasion and mapping of tumor heterogeneity
Vascularization and perfusion	Absent	Passive diffusion	Enable vascularization modeling	Enables engineered vascular network and enhanced perfusion
Operational cost	Low	Moderate	High	High initial cost for specialized infrastructure and technical expertise
Reproducibility	High, well-established protocols	Moderate	Variable, dependent on biological source and culture protocols	High
Main applications	Early-stage screening	Mechanistic studies	Tumor microenvironment modeling and advanced therapeutic strategies	Advanced physiological modeling and therapeutic testing

It is extremely aggressive and with fast progression from horizontal to vertical growth phase [28]. Lentigo malignant melanoma accounts for 5% of all melanomas, appearing in sun-exposed areas of the skin and relates to sun exposure [29].

From a molecular biology perspective, melanoma progression is highly associated with a multistep process involving alterations and additive genomic changes in several cancer genes. The main genetic mutations for cutaneous melanoma are B-Raf proto-oncogene (BRAF), neurofibromin 1 (NF1), and neuroblastoma RAS viral oncogene homolog (NRAS), being related to primary tumors. These mutations are primarily involved in the activation of the MAPK (mitogen-activated protein kinase) signaling pathway, a key driver in the early stages of melanoma development [30, 31]. As melanoma progresses, additional pathways become involved, particularly those regulating cellular immortalization and cell cycle control. These changes collectively lead to cell cycle deregulation and enhanced tumor progression [32].

As a result, the frequent genetic mutation in the DNA sequence of important genes and epigenetic alterations, through the cytosine methylation of DNA regulatory regions, contributes to tumor and drug resistance from primary and/or acquired resistance mechanisms. The primary resistance in melanoma is related to both genetic and epigenetic alterations in the tumor cells, consequently influencing alterations in their microenvironment. In comparison, the acquired/adaptive resistance is the result of the tumor treatment, inducing genetic and epigenetic dysregulation that affect important signaling pathways driving tumor growth [33].

The development of the correct prognostic of melanoma is directly connected with cancer staging, the latter being crucial for the design of effective therapies. The staging of melanoma-type skin cancer has been defined by the American Joint Committee on Cancer (AJCC), which is based on the assessment of the primary tumor and the presence or absence of regional lymphatic and distant metastases, together separating patients into four stage groups, as presented in Table 2 [34, 35].

Table 2 Melanoma staging based on the American Joint Committee on Cancer (AJCC) guidelines

Stage	Thickness	Description
IA	≤ 1 mm	Primary lesions, without ulceration of the overlying epithelium or invasion of the reticular dermis or subcutaneous fat
IB	≤ 1 mm	Primary lesions, with epithelial ulceration or invasion of the reticular dermis or subcutaneous fat
IIA (1)	> 1 mm and ≤ 2	High-risk primary tumors. Lesions with ulceration of the overlying epithelium
IIA (2)	> 2 mm and ≤ 4	Lesions without epithelial ulceration
IIB (1)	> 2 mm and ≤ 4 mm	Lesions with epithelial ulceration
IIB (2)	> 4 mm	Lesions without ulceration
IIC	> 4 mm	Lesions with ulceration
III	ns	Lesions in the regional lymph nodes or the development of a melanoma deposit in the skin or dermis along the lymphatic vessels before reaching a lymph node (transit or satellite metastasis)
IV	ns	Metastases indicated. M1a is limited to distant skin, subcutaneous tissues, or lymph nodes; M1b involves lung metastases; and M1c involves all other visceral sites

Current therapies

The current standard procedure for melanoma treatment is surgical excision, and it is applied to patients in stages I–III B. To ensure complete removal of the malignant tissue, between 0.5 and 2 cm of the healthy tissue around it is removed, depending on the stage of progression [30]. While effective for localized tumous—depending on the location—the removal of larger sections of tissue can require skin grafting, which is associated with limitations like donor site morbidity [36, 37]. Furthermore, local excision does not address any spreading of the tumor to the surrounding lymph nodes, requiring additional surgical interventions for their removal—which is often insufficient to completely remove all tumor traces [38]. Combined, these limitations define the clinical need for advanced systemic therapeutic solutions, capable of accurately targeting melanoma cells and preserving the surrounding tissues in the instances where surgical excision is insufficient.

Immunotherapy has been recognized as a potent solution for cases, where systemic treatment is required, with FDA-approved therapies dating back to 1995 [39]. Targeting the issue of lacking T-cell activation, immunotherapy harnesses the innate pathways which prevent the development of t####umours in healthy tissues. In the context of melanoma treatment, the application of multi-checkpoint antibodies, blocking cell-surface inhibitory receptors of T-cells, such as Ipilimumab and Nivolumab, have shown response rates as high as 53%, and are now the standard immunotherapy procedure for patients with advanced melanoma [39–41].

Targeted therapies present an alternative systemic approach to treating melanoma, where small proteins – called as immunotoxins – are designed to interfere with mutated intra-cellular molecular targets, essential for cancer growth, progression, and metastasis. Mutations in the BRAF gene frequently occur in advanced melanoma, resulting in uncontrolled cell proliferation. The combination therapy utilizing BRAF inhibitors (such as vemurafenib) and MEK inhibitors (such as binimetinib) has become increasingly prominent in targeted treatment strategies, demonstrating high rates of treatment [42, 43].

Despite the considerable achievements in patient recovery and survival through both immunotherapy and targeted therapies, complete remission still observed on a comparatively rare occasion (10–20%) [39]. This statistic illustrates both the complexity of the resistance mechanisms of melanoma and the clinical need for developing more advanced therapeutic solutions to account for them [40, 41].

Non-targeted approaches, such as chemotherapy, are also employed in melanoma treatment, especially in cases of relapse or when palliative care is indicated. Currently, dacarbazine remains the first and only FDA-approved chemotherapeutic agent for melanoma. Clinical trials involving dacarbazine have demonstrated modest response rates of approximately 10–20%, predominantly partial and typically not sustained over time. Additionally, significant adverse effects, such as nausea, vomiting, and myelosuppression, have frequently been reported with its administration. Consequently, the use of chemotherapy in melanoma has progressively declined due to the absence of novel chemotherapeutic agents that offer improved efficacy or reduced toxicity [17, 26].

Modeling skin melanoma: in vitro models

One of the most prominent challenges in the creation of new anticancer therapies is the development of a precise model that mimics the fundamental interactions observed in vivo. Tumor heterogeneity represents the most prominent roadblock in the implementation of effective therapies [44]. Malignant tumors exhibit inter- and intra-tumor heterogeneity, where inter-tumor heterogeneity represents the composition and organization observed in a type of cancer between patients and inter-tumor heterogeneity is related to the molecular and cellular heterogeneity, including clonal diversity and the tumor microenvironment (TME) [45].

Over the last few years, cancer modeling has been carried out using 2D cell cultures and laboratory animals [46]. However, even though 2D culture still has its benefits, such as simplicity of method and low cost; tumor tissue is not mimicked with complexity. The use of animals is limited to cost, controllability, reproducibility and design flexibility. The use of 3D models aims to bridge the gap between 2D cell culture and the use of animals in complex models [46].

Several 3D models have been built to overcome the limitations observed in 2D cell culture, such as spheroids, organoids and 3D bioprinted tissues, as presented in Fig. 1. These techniques provide more precise representations of tumor architecture, cellular interactions with the ECM, and biochemical gradients, being a superior research platform for drug discovery, tumor development and the development of new therapies [47]. Spheroids are the simplest model using 3D architecture, where the cells self-assemble to form with compact and spherical structures, developing signaling similar to those found in solid tumors. Organoids, on the other hand, are 3D structures that closely mimic the architecture and function of specific organs or tissues, being usually produced from tissue-specific cells or stem cells, requiring ECM and growth factors for the development [48].

In the context of melanoma research, several studies have developed and utilized spheroid models using different cell lines (WM1617, B16F10, MCM DLN) to investigate

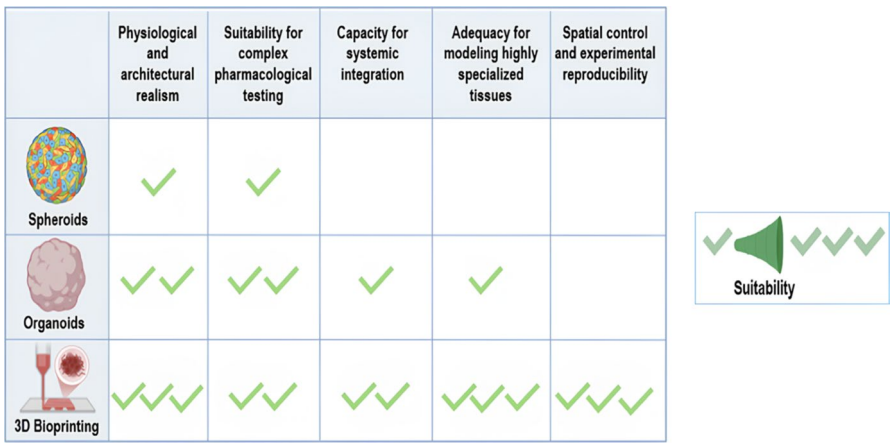


Fig. 1 Comparative assessment of spheroids, organoids, and 3D bioprinting technologies assessing melanoma modeling in vitro. Parameters include physiological and architectural realism, suitability for pharmacological testing, systemic integration, modeling of specialized tissues, and spatial/experimental reproducibility. Green checkmarks indicate increasing suitability, as represented in the scale on the right

tumor characteristics, therapeutic responses, and molecular profiles [49–52]. These models have proven valuable for studying tumor progression, cell–cell and cell–matrix interactions [49–52], and for evaluating treatment strategies such as photothermal therapy (PTT) applying advanced drug delivery systems, such as lipid nanocapsules [49–52] and the use of bioactive compounds like quercetin [49–52]. Comparisons between 2 and 3D cultures revealed significant differences in cell growth, metabolism, and gene expression, highlighting the relevance of 3D spheroids as more physiologically relevant models for understanding melanoma behavior and resistance mechanisms [49–52].

The use of organoids is also documented, demonstrating an advancement compared with spheroids, associated with the higher complexity of the tumor microenvironment. Studies have demonstrated the development of melanoma organoids using patient-derived tissue, highlighting the importance of organoid technology as a promising and biologically relevant approach. The results presented the understanding of melanoma progression, treatment response [53, 54], and resistance mechanisms [53, 54] contributing to the advancement of more effective and personalized therapeutic strategies. Additionally, organoids show important mutations presented in melanoma progression that are related to recurrence after surgery and therapy resistance, observing the effect of therapies, such as immunotherapy [53, 54].

Despite the improvements observed in the use of spheroids and organoids in melanoma research, achieving a complete recapitulation of the in-vivo environment and disease progression remains a challenge. Although they mimic aspects of the tumor microenvironment, missing in traditional 2D cultures and, to some extent, animal models, improvements are required [55]. Key challenges involve ensuring the accurate recapitulation of the multilayered and multicellular skin architecture, a structurally complex tissue that includes components such as the dermo-epidermal junction and skin appendages, and variation between individuals, due to parameters such as anatomical site, pigmentation, and aging [56].

The inclusion of the tumor microenvironment (TME) (specifically, stromal and immune components) in spheroids and organoids remains a challenge, due to the incomplete recapitulation of the native ECM, as well as the challenges in incorporating relevant immune cell subpopulations. Furthermore, weak vasculature and lymphatic networks limit long-term viability and modeling of important functions like angiogenesis and metastasis [57]. In this context, 3D bioprinting offers an effective solution for engineering complexity and intricacy into in vitro 3D models of melanoma, allowing the precise spatial organization of multiple cell types, such as immune cells, fibroblasts, and endothelial cells, within a defined ECM thought the use of specific bioinks and printing technology, presenting a model that closely mimics the native TME, by simulating the growth, diffusion, invasion and metastasis process that occurs in melanoma cells [58].

3D Bioprinting for melanoma modeling

Three-dimensional (3D) bioprinting has emerged as a transformative biofabrication technology that enables the spatially controlled deposition of living cells, biomaterials, and signaling molecules to construct complex tissue-like structures. Unlike conventional 2D and even traditional 3D culture models, 3D bioprinting offers precise architectural

control and enhanced biological relevance, allowing the recreation of tissue-specific microenvironments that more accurately mimic the in vivo tumor setting [58–61].

In the context of cancer research, and particularly melanoma, bioprinting allows for the generation of tissue constructs that reflect the heterogeneity and complexity of the tumor microenvironment (TME) [62]. This includes the ability to replicate spatial organization, cell–cell and cell–matrix interactions, and mechanical properties crucial for tumor progression and therapeutic response [63]. The bioinks are key to this process, being the component which encapsulates the cells and plays an integral role in the development of a native-like ECM [64]. These bioinks can be derived from a wide range of sources, including animal-derived proteins like collagen, gelatin, fibrin, and hyaluronic acid; plant-derived polysaccharides such as alginate and agarose; and synthetic polymers like polyacrylamide; together representing the range of properties that 3D bioprinted models can assume [64, 65].

Over the last years, the interest in the development of bioprinted artificial skin has significantly increased. Human skin equivalents (HSEs) are being developed though different bioprinting technologies, including vat polymerization, inkjet, laser-assisted, and extrusion methods, with differing degrees of structural, material, and cellular complexity. Recent advances have enabled the construction of multilayered in vitro skin models incorporating diverse cell types such as fibroblasts, keratinocytes, melanocytes, endothelial cells, adipocytes, pericytes, stem cells, and iPSCs [30, 66–68]. Beyond validating 3D bioprinting's co-culture capabilities, a range of studies report on the resulting models' capacity to generate structures, like vascular channels [69]. In the context of in vitro melanoma modelling, the development of functional vasculature in 3D bioprinted models presents an opportunity to observe not only the potential invasion of cancer cells through the interface with the healthy tissue, but also through the vascular walls, essentially metastasizing in vitro [70]. Immunological competence is another area, where 3D bioprinted models have shown promise, with both indirect activation of macrophages and direct activation of macrophages and T-cells, integrated in the constructs, having been reported [71]. In the context of the in vitro modeling of melanoma, these studies set the foundation for mimicking the immunological interplay which defines processes like the oncogenic activation of melanocytic nevi and the progression of localized melanomas and their transition to a metastatic phenotype [30, 66–68, 72].

The development of melanoma models remains modest. One of the major challenges is the development of experimental models that accurately replicate the diverse features of the TME, which trigger in-vivo drug resistance and patient-specific therapeutic responses. This complexity arises from the intrinsic properties of melanoma cells, including high genetic instability, phenotypic plasticity, the capacity to dedifferentiate into multiple states and the high level of metastasis [48, 73, 74]. Even so, 3D bioprinting represents a significant advancement in the development of high-fidelity melanoma models, offering a powerful tool for investigating tumor biology and evaluating emerging drug delivery systems with higher translational relevance.

Bioprinted melanoma models

Recent advances in 3D bioprinting have been increasingly related to the development of functional melanoma models that replicate not only the TME but also its interaction

with the surrounding skin microenvironment. The most recent research and the current status is reported in Table 3, assessing the cell types used and important information about the 3D bioprinted models, such as bioink composition, bioprinting technology and the outcomes of the work.

Recent studies highlight the use of different bioink compositions and cell combinations to construct melanoma models that better replicate the complexity of native tissue architecture and tumor interactions. These models incorporate a wide range of cell types, including established melanoma lines (e.g., A375) [77], patient-derived cells [78], fibroblasts, keratinocytes, endothelial cells, and stem cell populations [76], being co-cultured within specific bioinks, such as decellularized extracellular matrices, collagen, alginate–gelatin blends, and nanocomposite hydrogels. Such variability allows for the engineering of models that not only mimic the structural organization of the skin (epidermal, dermal, and hypodermal layers) but also simulate important features of the TME, including vascularization, immune modulation, and cancer-stem cell interactions [79]. Perhaps the most critical aspect of the 3D bioprinting process emerges as the interplay between the selected bioprinting technology and the bioinks with suitable physicochemical and biological properties, both of which are essential for constructing functional and physiologically relevant models [80, 81].

Bioinks

The term bioink refers to a cell-laden formulation suitable for processing via biofabrication or 3D bioprinting technologies, which may also include biomaterials and bioactive molecules [82]. These formulations are designed to closely replicate the native in vivo conditions, including those of the tumor microenvironment, to support tissue-specific architecture, cell viability, and functional behavior [82, 83]. Hydrogels are widely utilized in bioink development due to their highly hydrophilic nature, enabling efficient water absorption and retention, closely mimicking the hydrated state of the native ECM [84]. The choice of bioink and its crosslinking reaction are fundamental in the resulting biomatrix properties, such as biocompatibility (e.g., cell viability and proliferation); printability (e.g., shape fidelity and rheology); biomechanics (e.g., resemblance) and biodegradation (e.g., tissue formation) [62].

Natural biomaterials are highly used in bioink development. Mimicry of the native skin ECM has proven a potent approach, with both polysaccharides mimicking its glycosaminoglycan component – such as chitosan, collagen, hyaluronic acid, and alginate – and proteins, approximating its fibrillar structure – like collagen type I and gelatin – exhibiting high levels of biocompatibility and adaptability [85, 86]. Collagen type I, is the predominant structural protein in the dermal ECM, providing essential biochemical signals and supports cell adhesion, proliferation, and differentiation, making it a fundamental component in skin tissue engineering [87]. Its fibrillar structure closely resembles native tissue architecture, facilitating the development of physiologically relevant skin models [88]. Previous studies have explored the potential of alginate combined with gelatin modified via partial oxidation, resulting in the so-called alginate-dialdehyde gelatin (ADA-GEL), promoting the improvement of the biocompatibility and printability [83].

Synthetic biomaterials including polyethylene glycol (PEG), PCL, polyvinylpyrrolidone (PVP), poly(l-lactic) acid (PLA), and poly(lactic-co-glycolic) acid (PLGA) are highly

Table 3 Summary of recent 3D bioprinted melanoma models highlighting key parameters including cell types, bioink composition, bioprinting technology, and main outcomes

Study	Cell type(s)	Bioink composition	Bioprinting technology	Model features/outcomes	Application/ urpose
Paula Vázquez-Aristizabal et al. [75]	Melanoma and fibroblast cell lines (451Lu, WM164, HDFs and Hs27)	Decellularized extracellular matrices (dECMs)	3D pneumatic extrusion-based bioprinting	3D metastatic co-culture melanoma model, recapitulating features of both the tumor and the skin microenvironment	Tumor microenvironment simulation and drug screening
Schimid et al. [63]	Melanoma and adipose-derived mesenchymal stem cells (Mel Im and ADSC)	Alg/HA/Gel (0.5% alginate, 0.1% HA, 3% gelatin)	Extrusion-based 3D printing	3D melanoma model tested as a new vascularized model; viable for drug testing; multi-cellular interaction observed	Drug testing and tumor–skin interactions
de Andrés et al. [76]	Malignant melanoma (MM), Endothelial cells, keratinocyte, stem cells, fibroblasts, cancer stem cells and co-culture (A375, HUVECs, KTCs, Mel-1, MSCs, FBs, CSCs, HUVECs-FBs)	Matrix of collagen type I and agarose	Extrusion-based 3D printing	3D bioprinted human melanoma model recapitulating the TME and the skin layers (epidermal, dermal and hypodermal) with vascularization. Further implemented in vivo experiments, analyzing the development of MM	Personalized platform for cancer testing
Teixeira et al. [77]	Melanoma cells (A375)	Pectin, nanofibrillated cellulose and lysozyme amyloid nanofibrils	Extrusion-based 3D printing	Bioprinted constructs with controlled stiffness and nanofibrillar nanocomposite pectin hydrogel bioinks	Mechanobiology of melanoma with a new bioink for drug screening
Jeong et al. [78]	Patient-derived melanoma explants	Collagen	Extrusion-based 3D printing	3D printed collagen scaffolds enhance the viability and preservation of the explants, compared to traditional 2D cultures	Tumor microenvironment simulation

used in 3D bioprinting due to their affordability, batch-to-batch consistency, and superior structural stability, which contribute to excellent printability. However, their lack of intrinsic bioactivity and native cell-interactive cues often results in limited biocompatibility, necessitating chemical modification or blending with natural biomaterials to support cell adhesion and function [89].

Considering the development of skin and melanoma models, selecting appropriate bioinks for 3D bioprinting presents significant challenges due to the intricate architecture of skin and the complex interactions within the tumor microenvironment. Hydrogel-based bioinks are widely used in the development of 3D bioprinted skin and melanoma models, presenting key elements as extrudability, shape fidelity, and bioprinting accuracy, being often combined with relevant materials for the ECM development, such as keratinocytes (KCs), melanocytes (MCs) and fibroblasts [90]. Therefore, the selection and optimization of bioinks must consider not only for mechanical and printability requirements but also for their capacity to support multicellular interactions and accurately mimic both the healthy skin architecture and the pathological progression of melanoma [91].

Bioprinting technologies

3D bioprinting technologies have established themselves as powerful tools for mimicking native tissue architecture for both tissue engineering and cancer modeling, allowing the precise deposition of cells, biomaterials, and signaling molecules in complex, multicellular models [92, 93]. The most common approaches for skin and melanoma development include extrusion-based, inkjet, laser-assisted, and lithographic-based bioprinting, each with its respective advantages and limitations relating to cell viability and printing fidelity [30].

Extrusion-based bioprinting is well-suited for the in vitro printing of tissues and organs, due to its combination of cost-effectiveness and precision in the bioprinting material deposition [94]. It employs extrusion forces to precisely deposit bioinks on a platform, normally using a disposable plastic syringe, and the dispenser can be either pneumatically (compressed air) or mechanically (piston- or screw-driven). One of the major advantages is the ability to print viscous fluids with high cell densities. Its limitations relate to its inferior resolution (200–1000 μm), possibility of nozzle clogging, and shear stress leading to decrease in cell viability [90, 95].

Inkjet, or drop-on-demand bioprinting, dispenses small bioink droplets using a layer-by-layer process in a platform, being a low-cost technique with high speed and resolution (20–100 μm). The process uses different actuation mechanisms to eject bioink droplets: thermal systems generate pressure via localized heating, piezoelectric systems apply mechanical pulses through actuators, and electric field-based systems use electrostatic forces to drive droplet formation into a crosslinking medium. Its main limitations are associated with low vertical printing fidelity and nozzle clogging when viscous materials are used [81, 95].

Laser-assisted bioprinting utilizes a laser source, a donor slide (usually a glass slide coated with an energy-absorbing layer and a thin film of bioink), and a receiving substrate. When the laser is irradiated at the metal layer's focal point, it causes localized energy absorption, leading to evaporation and bubble expansion, generating a shockwave

and jet that propels the bioink droplet in the receiving substrate. One of its key advantages is the ability to process a broad range of bioinks, including those with high viscosities and cell densities, without the risk of nozzle clogging. This functionality and the high printing resolution it can achieve put it ahead of extrusion- and inkjet-based bioprinting systems. However, high cost and the limited height of the constructs it can achieve, as well as reproducibility and thermal damage (nano-second laser irritation) limitations, hinder the technology's large-scale potential. [81, 90, 96].

Lithographic bioprinting (stereo lithography (SLA), digital light procession (DLP) and two photon polymerization (2PP) use specific wavelength and light intensities to polymerize the bioink in a 3D structure. Advantages involve high printability precision and versatility, but the exposure to UV light leading to cell damage and the prolonged fabrication times in layer-wise methods remains as major limitations [90, 96, 97].

Among bioprinting technologies, extrusion-based bioprinting is highly used for skin and melanoma models. The advantages related to printability of viscous fluids with high cell densities, is essential for replicating the stratified structure of skin and the complexity of the tumor microenvironment. Additionally, the development of multilayered models is supported by the deposit of continuous filaments, allowing the deposition of keratinocytes, fibroblasts, melanocytes, and melanoma cells. Although inkjet and laser-assisted methods offer higher resolution, the limitations associated with high-viscosity bioinks, and scalability reduce their suitability for the fabrication of multicellular constructs [90].

Novel therapeutic applications of photodynamic therapy and drug delivery systems for melanoma treatment in 2D and 3D models

The association of PDT and advanced drug delivery systems has emerged as a promising strategy for enhancing therapeutic efficacy in cancer treatment, including melanoma [98]. PDT offers a minimally invasive treatment, involving the activation of chemical agents, photosensitizers, with light a specific wavelength and molecular oxygen (O2) to generate reactive oxygen species (ROS) inducing localized cytotoxic effects [99]. However, despite the therapeutic potential of PDT, its application in melanoma involves further consideration, due to limitations to the light conductivity of the TME [100]. Melanin absorbs in the 500 to 600 nm spectral range and can block the penetration of the light used to activate most photosensitizers [101]. In addition, melanoma has intrinsic defense mechanisms, such as drug efflux and antioxidant capacity, negating any ROS-mediated oxidative damage; together limiting the efficiency of PDT [102, 103].

Multiple strategies have emerged in the context of overcoming PDT's limitations in melanoma, exploring novel strategies and combined therapies to enhance the efficacy of PDT. Among those, targeting for melanoma biomarkers, such as tyrosinase, has been done successfully by conjugating targeted inhibitors to the photosensitizer molecule [104]. The targeting strategy improved cellular uptake, increased singlet oxygen production, and achieved greater phototoxicity under laser irradiation, compared to non-targeted compounds, presenting an effective strategy in the typical resistance of melanoma to PDT [104].

Molecular design of porphyrins has also shown promise, with longer chains appearing to target melanoma cells when compared with cationic pyridinoporphyrins, free

and complexed with Zn (II) [105]. The strategy showed the optimization of the photosensitizer, overcoming poor tumor selectivity, suboptimal photosensitizer (PS) delivery, optimizing the effectiveness of PDT for the treatment of melanoma in different human malignant melanoma cell lines (A375 and MeWo) [105].

Despite advances in PDT in recent years, the therapeutic efficacy can still be limited by the low solubility of PS, leading to low bioavailability and inefficient activation in heterogeneous tumor environments. To overcome those limitations, researchers have increasingly focused on integrating PDT with nanocarrier-based delivery platforms, including liposomes, nanoemulsions, polymeric nanoparticles, and hydrogels, to improve photosensitizer bioavailability, control release kinetics, and enable targeted delivery [106]. This combinatorial approach not only enhances PDT precision but also opens new possibilities for co-delivery of chemotherapeutics or immunomodulators, offering interactive anti-tumor effects and reduced systemic toxicity [106].

In the context of improving photosensitizer delivery and selectivity for PDT, liposomal systems and oil-in-water nanoemulsions have emerged as promising strategies for melanoma treatment. Studies have demonstrated favorable results with the use of liposomes [107, 108] and nanoemulsions [109] loaded with hypericin—a natural compound with key properties for light activation, permitting application in PDT and the treatment of melanoma. The studies were conducted using 2D models (B16-F10 cell line) [108] and more complex models, such as three-dimensional (3D) melanoma model (coculture of B16F10 and Balb/c 3T3 cells), better mimicking the tumor microenvironment than conventional 2D cultures [109].

Additionally, researchers explored combined therapies in which nanoliposomes encapsulate galectin-3 to stimulate immune activation [110]. This approach recruits natural killer (NK) cells to melanoma tumor sites after PDT, offering a combined strategy against melanoma's therapeutic resistance [110].

Following on the advances of new drug delivery systems PDT in melanoma, the use of lecithin-based invasomes as nanocarriers for 5-aminolevulinic acid (5-ALA) was also explored, using advanced 3D melanoma models as platform of research [111]. While 5-ALA is a well-established photosensitizer in PDT, it presents limited ability to cross skin barriers facing challenges for topical application. The invasome formulation demonstrated improved diffusion, high encapsulation efficiency, and enhanced reactive oxygen species generation after light activation. Importantly, the study, presented the combination of a 3D melanoma model with advanced drug delivery systems as a relevant platform for optimizing topical PDT strategies and better predicting therapeutic outcomes.

In general, the combination of PDT into melanoma treatment strategies has shown significant potential, in particular when combined with advanced drug delivery systems such as nanocarriers [101]]. These systems can improve the accumulation of photosensitizers in tumor tissues, enhance penetration, and ultimately increase therapeutic efficacy [108, 110].

Despite the emergence of novel and promising developments in PDT drug delivery for melanoma treatments, their further development is directly dependent on their validation and optimization for efficacy in the tumor microenvironment. In this context, the integration of invitro platforms with advanced physiological relevance, such as three-dimensional constructs and 3D bioprinted models of higher complexity, present

a valuable opportunity to streamline and accelerate PDT-mediated drug development. While three-dimensional cast constructs recapitulate melanoma and skin cell interactions in space, they are associated with limitations in their ability to establish controlled interfaces between the cell types of interest, thus hampering researchers' ability to study inter-cell type interactions, as well as cancer invasion [112]. In this context, 3D bioprinting evolves in vitro models, with its functionality to establish defined layers and compartments for different cell types, permitting in vitro macroscopic mimicry of both healthy and melanoma-affected skin structure in all its stages [113]. In capturing the macroscopic arrangement of the native tissue, these models allow for the construction of more robust systems, in terms of tumor architecture and differential cellular and extracellular matrix composition: together, an environment which captures the native melanoma microenvironment more faithfully. Applied in the context of PDT therapeutics development and optimization, these advanced models bridge the gap between preclinical research and clinical translation, paving the way for more accessible and effective PDT-based melanoma therapies [48, 111].

Challenges, limitations, and future perspectives of bioprinting melanoma models

The development of 3D bioprinted models of melanoma remains a challenge, due to the complexity of mimicking the TME with sufficient accuracy, as well as its heterogeneity and microenvironmental interactions, including immune, stromal, and vascular. Additionally, these models frequently lack functional vascularization, limiting long-term viability and drug diffusion. In the therapeutic context, integrating PDT and drug delivery systems introduces further limitations [114, 115]. These include insufficient light penetration into deep tissues, oxygen dependency of PDT in often hypoxic tumor regions, and the poor solubility and bioavailability of many photosensitizers. However, the use of drug delivery systems has been gaining prominence, since they can improve the stability, selectivity, bioavailability, and photodynamic efficiency of photosensitizers, overcoming the cited limitations [116–118].

Most studies of PDT and drug delivery systems for melanoma are still based on two-dimensional (2D) models, which do not replicate accurately in tumor environment, lacking the required complexity [57, 119]. In this context, the development and use of three-dimensional (3D) models in recent years has shown that more complex models are more suitable for evaluating the efficacy of nanocarriers in tumor treatment studies. 3D bioprinting overcomes key challenges in melanoma modeling by enabling the controlled spatial organization of cells and biomaterials, mimicking the tumor's architecture and microenvironment with higher accuracy [16, 120]. This includes replicating heterogeneity, ECM structure, and introducing vascular-like characteristics to improve oxygen and drug diffusion: all essential for evaluating PDT and drug delivery systems and allowing better drug screening in preclinical studies [57]. Despite this, studies continue to report technical challenges, such as variability between batches (volume, shape and density) and difficulty in standardizing and incorporating elements such as blood vessels and immune components [121, 122]. The addition of complex components, such as immune cells, can represent a breakthrough strategy in the development of complex bioprinted melanoma models, once immune cells play a critical role in the tumor microenvironment,

showing direct effect on the tumor progression and resistance, due to the transformation of immune cells to a tumor-resistant phenotype, leading to significant advance and understanding of therapy outcomes [123, 124]. Although the combined use of PDT, drug delivery systems, and 3D bioprinted models presents a promising frontier for the study of prospective melanoma therapies, the techniques integrating all these approaches are yet to demonstrate their full potential.

Another major hurdle for bioprinting technologies is the absence of specific and comprehensive frameworks to regulate their clinical translation [125]. Currently, no global regulatory authority has established precise standards for products involving cells and tissues. Only non-biological 3D printed devices are covered by the 2016 U.S. FDA guideline, Technical Considerations for Additively Manufactured Devices. Devices containing live cells require different regulatory pathways, which it does not cover [125, 126]. However, the use of 3D bioprinted models align with the evolving regulatory landscape, where agencies like the U.S. FDA and EMA are actively encouraging the use of complex in vitro models (CIVMs) to reduce reliance on animal testing in line with the 3Rs principle [127].The FDA's Predictive Toxicology Roadmap and New Alternative Methods (NAMs) strategy emphasize the integration of human-relevant in vitro systems for toxicity, efficacy, and mechanistic studies [127, 128]. Furthermore, there is increasing recognition that well-characterized bioprinted tumor models can serve as valuable complementary tools in investigational new drug (IND) submissions, especially when they demonstrate physiological relevance and batch-to-batch consistency [129]. While challenges remain, such as standardization, validation, and regulatory classification, the translational potential of bioprinted melanoma constructs is substantial and growing [125, 126].

Additionally, recent advances range across printing human-scale tissues with volumetric printing, moving away from synthetic biomaterials and toward low-viscosity functional biomaterials, incorporating vascular networks, and using multiple cell types to mimic native tissue complexity [130]. However, limitations related to construct stability during maturation, cell viability over the long term, and limitations of methods like volumetric bioprinting are significant concerns [94]. As such, the development of materials which can promote adhesion, proliferation, and the development of native tissue features are urgently needed [131]. Despite these limitations, clinical trials are ongoing in the fields of dentistry, orthopedics, and maxillofacial surgery. These trials are exploring topics like dental prostheses, bone implants, and orthopedic devices [125], together illustrating the momentum that the field is gaining.

Conclusions

Malignant melanoma remains as the most aggressive skin cancer type, presenting several challenges related to its treatment due to progression and mutation complexity. At the same time, the development of reliable models to mimic the tumor microenvironment intricacy exhibited in cancer tissues remains as one of the major limitations. Considering the advances in the development of tissue engineering and 3D bioprinted models, new technologies are being explored for the development of complex models, successfully recapitulating essential tumor microenvironment features in melanoma [75, 77]. Linked to this, the improvement of new therapies such as the association of PDT

and nanotechnology are being discovered as key strategies to overcome problems related to traditional treatments for melanoma, decreasing side effects and the need for surgery, in addition to not presenting acquired resistance. Taken together, these advances highlight the transformative role of 3D bioprinted models as predictive platforms for preclinical testing, bridging the gap between conventional in vitro systems and clinical translation. By integrating multiple cell types, ECM components, and vascular-like networks, these models allow more accurate evaluation of therapeutic responses and resistance mechanisms. Moreover, the convergence of bioprinting with innovative therapies, such as nanocarrier-based drug delivery systems and PDT, provides new perspectives to enhance selectivity, reduce toxicity, and improve patient outcomes. Despite current challenges related to model standardization, vascularization, and regulatory pathways, the synergistic use of 3D bioprinting and advanced therapeutic strategies holds great promise for the future of melanoma treatment, paving the way for more effective, personalized, and clinically translatable solutions.

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Author contributions
S.R.d.A. and A.P.A.: writing—original draft, formal analysis, data curation. D.C.M.d.S.: writing—original draft. I.F.d.P.: writing—original draft. M.L.F: writing—original draft. L.M.G.: writing—review and editing. F.L.P.: writing—review and editing, funding acquisition, project administration, resources, formal analysis, supervisor. All the authors reviewed the manuscript.

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Data availability
No datasets were generated or analyzed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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