Biomaterials for Mimicking and Modelling Tumor Microenvironment

6

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Abstract

This chapter summarizes the current biomaterials and associated technologies used to mimic and characterize the tumor microenvironment (TME) for developing preclinical therapeutics. Research in conventional 2D cancer models systematically fails to provide physiological significance due to their discrepancy with diseased tissue's native complexity and dynamic nature. The recent developments in biomaterials and microfabrication have enabled the popularization of 3D models, displacing the traditional use of Petri dishes and microscope slides to bioprinters or microfluidic devices. These technologies allow us to gather large amounts of time-dependent information on tissue–tissue, tissue–cell, and cell–cell interactions, fluid flows, and biomechanical cues at the cellular level that were inaccessible by traditional methods. In addition, the wave of new tools producing unprecedented amounts of data is also triggering a new revolution in the development and use of new tools for analysis, interpretation, and prediction, fueled by the concurrent development of artificial intelligence. Together, all these advances are crystalizing a new era for biomedical engineering characterized by high-throughput experiments and high-quality data.

Furthermore, this new detailed understanding of disease and its multifaceted characteristics is enabling the long searched transition to personalized medicine.

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Here we outline the various biomaterials used to mimic the extracellular matrix (ECM) and redesign the tumor microenvironment, providing a comprehensive overview of cancer research's state of the art and future.

Keywords

Tumor micro environment (TME) · Biomaterials · Microfluidics · Extra cellular matrix (ECM) · 3D models · Cancer

6.1 Introduction

The disease cancer was named after the word crab by the father of medicine, Hippocrates, is caused by consecutive mutations in the DNA sequence initiating from a single abnormal cell. The progressive gene instability with a few rounds of selective mutation leads to the formation of a tumor mass [1]. After several cycles of cell division, the expanded mass of neoplastic cells breaks open the basal membrane to invade locally by metastasizing and eventually causing fatality. The nature of the tumor and its progression depend on external and internal environmental factors. The contributing factors occur in parallel or sequence, leading to a massively growing lump of cells showing abnormal and uncontrolled clonal expansion [2]. Other important aspects like cell adhesion, secretion of proteolytic enzymes, tumor angiogenic factors, etc., are increasingly studied to understand the nature of tumor progression and its ability to escape the immune system and resist drug therapies [3]. The most common therapies to treat cancer consists of chemo and radiotherapy along with checkpoint inhibitor drugs. The drugs have shown efficacy but with many side effects on healthy cells, highlighting the central role of biomaterial research in providing better models for more targeted and personalized therapies [4, 5].

In this context, materials with tunable properties matching tissue stiffness give us a fair chance to study cancer cell development, progression, and infiltration properties in real-time outside the "noisy" in vivo environment. Here we cover the biomimetic use of biomaterials to reproduce the tumor microenvironment for the study of tumoral development and immunotherapy.

6.2 Cancer Pathology and Progression

Despite the vast number of open questions related to cancer, its conceptualization as a genetic disorder and a somatic mutation was established more than a century ago [6]. Due to the shift in the chromosomal stability, the transition from normal to malignant tumor is witnessed. Nonetheless, be it malignant or benign still ends up killing their host. Therefore, understanding the mechanisms behind the disease development and progression is central to developing new therapies. In particular, the prevention of tumor dissemination from the origin to distant sites, which might

occur before diagnosis, is necessary to enable targeted treatments [7, 8]. The tumoral extravasation to distant sites starts with an epithelial to mesenchymal transition, followed by the invasion into the new site by degrading the basal lamina of the ECM. In the new site, the cells are characterized by their reliance, enabling them to survive long enough to promote angiogenesis and start expanding. After the process is complete, the new tumor will continue developing while evading the immune system [9].

In the following section, we describe the complexity of the tumoral niche and the complex relations with the microenvironment enabling cancer persistence.

6.2.1 Cancer Complexity and Dynamics

The robustness of tumors results from complex functionalities and synergetic mechanisms, enabling cells to continue proliferating against checkpoints and anti-cancer therapies. This robustness is theorized to be connected to an outstanding feedback control system that allows rapid adaptation and functional redundancy to replace compromised functionalities.

6.2.1.1 Functional Redundancy

Cellular redundancy plays an essential role in maintaining cancer cells' robustness, a strategy used in cellular biology at many levels [10]. For example, it has been seen that the transcription factor E2F1-3 is involved in many functions like DNA replication, mitosis, DNA repair, differentiation, etc., and also restricts the mammalian cells from entering the S-phase [11]. Additionally, Cdk2 compensates for Cfk-4, -6, and -1 due to their redundancy in governing the cell cycle [12]. In the case of cancer cells, numerous overlapping changes in their genes ensure their survival and resistance to drugs through the escape of cell cycle checkpoints, uncontrolled growth, dodging apoptosis, etc. These mechanisms effectively enable, among others, high drug efflux, decreased drug uptake, and very efficient DNA repair. This sophisticated, rapid, and mostly unknown response to external factors makes the treatment of advanced cancer largely ineffective [13]. The concept of biological redundancy is always neglected while drug designing procedure which thereby leads to the development of multidrug-resistant cells due to the dysfunctional cell cycle mechanism [14]. This translates into an acquired cancer cell resistance, even before their exposition to a new treatment.

6.2.1.2 Feedback Control System

The feedback loop mechanism and associated genes synchronously preserve the redundancy and robustness of the neoplastic cells [15]. When the feedback control is triggered, a cascade of reactions occurs to ensure the survival of cells. A paradigmatic example of this system is the lowering of cytotoxin levels in the tumor cells, maintained by the upregulation of the multidrug-resistance 1 gene (MDR1) through a positive feedback mechanism. In this state, cells export drugs out of the cytoplasm through an ATP- dependent efflux pump [16]. Similarly, the

overexpression of the MDM2 gene due to internal changes causes degradation of the p53 gene, thereby blocking apoptosis of the cancer cell. MDM2-p53 interaction is generally enhanced once the DNA is seriously damaged, and the feedback control loop mechanism contributes to the robustness [17]. The feedback loops maintain the cell cycle against cellular perturbations, enabling the continuous proliferation of the neoplastic cells after therapy.

Along with the intracellular feedback loops, tumor cells also initiate these feedback mechanisms in response to the environmental cues. When the tumor cells harmonize with the extracellular matrix and the immune cells, they adapt to the innate tumor-suppression mechanism and cope with its environment. In instances of stress, such as nutrient deprivation or low oxygen concentration, tumor cells acclimatize to the change by either taking advantage of the swapped condition or migrating to a distant location, thereby starting the process of metastasis [18].

6.2.2 Tumor Microenvironment

The tumor microenvironment (TME) is one of the regulatory factors for tumor formation and progression. Therefore, monitoring the surrounding microenvironment, along with the genetic factors, is required to understand the dynamics of the neoplastic cells. The TME comprises the tumor cells, fibroblasts, endothelial cells, adipose tissue, specific immune cells, and proteins making up the extracellular matrix (ECM) [19]. The cancer cells use healthy cells to their advantage, signaling cross-talk mechanism to initiate and maintain tumorigenesis, metastasize and develop an effective resistance against therapeutic drugs [20].

6.2.2.1 Cancer-Associated Fibroblasts

Cancer-associated fibroblasts (CAFs) are healthy (i.e., non-cancerous) cells that are generally activated and rapidly multiply in wounds. However, in the case of a tumor, they are perpetually activated as a mechanism for the tumor to grow [21].

The activation is maintained by the plethora of secreted growth factors and chemokines, playing a crucial role in cancer progression and ECM remodelling. For example, CAFs have overexpression of Galectin-1 protein, which leads to a poor prognosis of the disease [22]. Additionally, it was seen that MMP-2 was a major remnant from the CAF-CM (conditioned media) derived from oral squamous cancer cell culture and thereby, leading to keratinocyte dis-cohesion and invasion into the epithelium when studied in collagen gel [23].

6.2.2.2 Immune and Inflammatory Cell

Immune cells are programmed to survey and defend bodily functions, eliminating invading foreign pathogens. During a typical (i.e., non-cancerous) illness, the immune cells do not persist at the site of inflammation, avoiding the creation of downstream pathologies. However, the immune-inflammatory cells linger in the inflammation site in abnormal cases, leading to neoplasia and angiogenesis, signaling the initial cancer stage [24]. After that, these cancer cells will transit through

three stages in coordination with the immune cells (i.e., elimination, equilibrium, and escape) [25]. In the initial elimination stage, the immune cells mitigate the diseased cells to defeat the budding neoplastic cells. However, in the consecutive stages, the cancer cells evolve phenotypic changes to continue growth and progression. In this limited environment, cancer cells continue developing using the support of the immune cells to escape and promote invasion. The interaction of various cell types with the inflammatory cells in the TME results in the immune cells behaving erratically, producing numerous cytokines and other proteins, thereby challenging the design of uniform drugs for therapy. Similarly, healthy granulocytes can contribute to cancer development by producing cytokines-like hematopoietic growth and granulocyte colony-stimulating factors [26].

6.2.2.3 Lymphatic Networks

The blood and the lymphatic networks are the major components helping the tumor progress and escape the defense mechanism [27]. This process happens in two main ways. First, a tumor surrounded by new blood vessels is generally leaky, twisted, and inefficiently retains its cells, leading to quick metastasis [28]. Second is the angiogenesis process, an omnipresent and necessary factor of tumorigenesis. The new vasculature significantly increments the complexity of the tumoral site, a drawback overshadowed by the provided ability to access oxygen from the surrounding environment [29]. Therefore, the new or primary tumor cells find it easy to adapt to hypoxic conditions with the help mainly of the blood system.

Similarly, the lymphatic system excludes the immune cells not to affect the survival of the tumor cells. The myeloid-derived suppressor cells (MDSCs) and the immature dendritic cells restrict T-cells' normal function to clear out the mutated cells [30]. Additionally, the lymphatic vessel forms a physical link between the sentinel lymph nodes and the primary tumor. This connection, in turn, creates the primary direct path for the tumor cells to spread to all possible other locations.

6.2.2.4 ECM in the Microenvironment

The extracellular matrix is made from various proteins such as collagen, laminin, and fibronectin and secretes multiple growth factors and cytokines, which contribute to cancer development and metastasis [31]. The extra cellular secretions affect the tumor progression by altering the phenotype of the stromal cells, causing many oncogenic mutations providing enhanced survival capacity in hypoxic or acidic environments. After these ECM-induced mutations, tumoral cells tend to express markers like TGF-ß1 and IL-6, which makes them escape the detrimental effect from the macrophages and results in a high rate of angiogenesis [32] (Fig. 6.1).

6.2.3 Emerging Biomaterials for Cancer Models

One of the critical aspects of cancer survival and development is its microenvironment. Understanding the tumor microenvironment and replicating it in vitro allows rapid and efficient designing of drugs for preclinical trials, providing a controlled

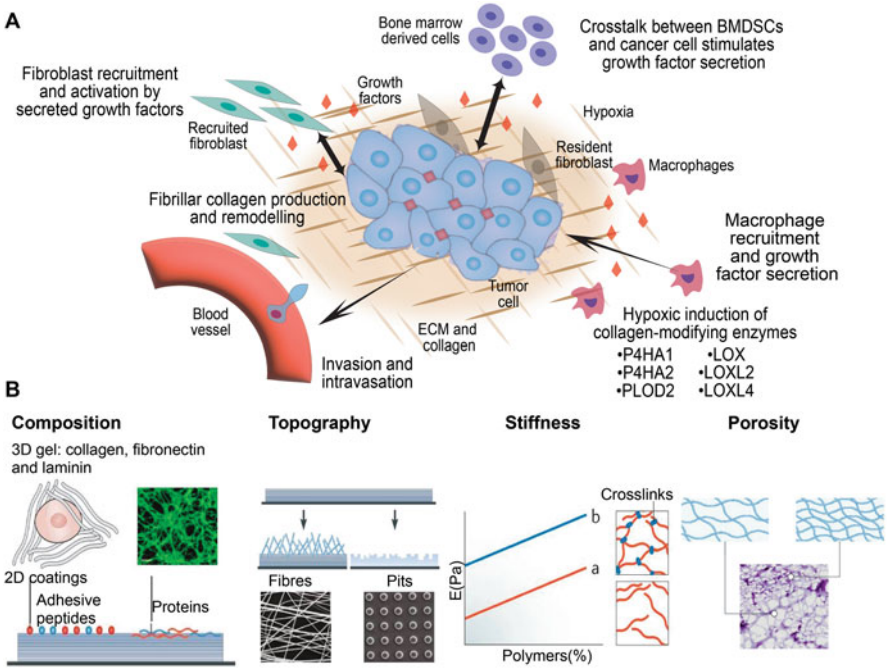


Fig. 6.1 Representation of the dynamic tumor micro environment (TME). (**A**) The illustration represents the cross-talk that takes between the normal cells and the mutated cells. Several growth factors are involved in the process and interplay of the microenvironment for tumor development and metastasis. (**B**) Modelling the TME by using materials to mimic the ECM composition, stiffness, topography to study cancer development (Reproduced with permission from [33])

environment for testing. This enhanced control is critical, as tumors are characterized by changing behavior and a heterogeneous population of cells, difficulty the interconnection between genetic and phenotypic observations. Among all the engineering tools developed to understand the disease progression, biomaterials have played a central role in the past two decades as vehicles to replicate and manipulate the cancer TME. The broad number of materials developed to encapsulate and maintain cells can be logically divided between those using the native components of organisms (i.e., natural) and those synthesized for the task (i.e., synthetics). The following section is a general overview of the main materials to engineer cell encapsulations and their use as scaffolds to study cancer.

6.2.3.1 Natural Matrix

Two-dimensional (2D) cell cultures are prevalent, and they are the first approach to model disease due to their simplicity in producing and characterizing cell populations [34]. However, this straightforwardness comes with the trade-off of a poor ability to replicate physiological behaviors and disease mechanisms. In that aspect, three-dimensional (3D) models significantly outperform their flat

counterparts. This ability to reproduce physiological mechanisms is greatly enhanced when in addition to reproducing the 3D geometry, the model is developed using native and naturally derived biomaterials replicating the composition.

Matrigel is one of the first commercial natural 3D models for cell culturing and still being among the most popular ones. Matrigel is a combination of collagen IV, entactin, perlecan, laminin, and other growth factors, and while it has broad use, it has gained special attention in cancer for the specific culture pancreatic cells. However, the main limitation of Matrigel, arising from its natural origin, is the batch-to-batch variation, constraining the reliability of its results when mimicking the ECM [35, 36]. Another popular option to study cancer cell migration in an environment close to the native one is the direct use of collagen type I and IV, the abundant protein molecule in the interstitial stroma [4, 37]. Collagen is denser than normal tissue and linearly organizes to provide an appropriate and tuneable matrix stiffness to culture cancer cells. It also allows secretion of MMPs by the cells, further influencing downstream cell–cell communication for cell migration, and promoting cell adhesion, thereby excluding the requirement to functionalize the matrix artificially [38].

An equally popular natural approach is the use of fibronectin, a cell surface glycoprotein binding to integrins. It supports cell invasion and metastasis, and it is known for having pro-tumorigenic factors. In a 3D cell culture setup, fibrillar fibronectin closely mimics the natural ECM, supporting spheroid cell cultures and cell migration [39].

Hyaluronic acid (HA), a glycosaminoglycan, has also been demonstrated to provide a very stable platform to culture cancer cells. HA binds to the receptor CD44, which encourages cancer cell proliferation. The material has been extensively used to explore the behavior of pancreatic carcinoma cells. Other properties of HA include initiating cell invasiveness, supporting spheroid formations, and enabling tweaking the scaffold stiffness [40].

Among other native materials with growing use as artificial scaffolds, laminin, and fibrinogen are rapidly gaining interest, as they have a demonstrated ability to mimic the properties of basement membrane along with enhancing cell invasiveness.

6.2.3.2 Synthetic Matrix

Synthetically designed polymers offer a broader range of biochemical and biomechanical properties over naturally occurring ones [41]. However, since they generally lack the moieties required for cell adhesion, invasion, and migration, they demand many added chemical modifications. In this category, the most popular options are polyethylene glycol (PEG), poly (vinyl alcohol) PVC, and polyacrylamide (PAAm). These molecules are conjugated with RGD groups or with MMPs to enhance their susceptibility to cells.

Modifying the matrix with peptides makes the material suitable to study the growth and morphogenesis of cancer cells. The degree of customization of synthetic polymers enables PEG-based hydrogels and oligopeptides-derived hydrogels with specific amino acid sequences. This customization enables the accurate mimicking of the TME for cancer research using chemically stable and versatile peptides. A

common example is the antiparallel organized peptide RADA-16, which has great implications in studying anticancer drug resistance [42].

A common approach to simultaneously have the customizability of synthetic polymer and the accuracy of natural ones is using hybrid hydrogels, developed by combining natural and synthetic polymers. The natural polymers act as the bioactive part, taking care of the cell adhesion and other biological phenomena. In contrast, synthetic polymers help regulate the mechanical properties, enabling the replica of the tissue counterpart. For example, norbornene modified gelatin and thiolated hyaluronic acid were cross-linked in the presence of white light with eosin-Y and used to culture pancreatic cancer cells [43]. The gel's stiffness is managed by controlling the cross-linking (resulting in the desired modulus). Similarly, collagen fibers chemically linked to (PEG-diNHS) alter collagen's stiffness without altering the space between the adjacent fibers, preserving the similitude with natural tissue. With a tensile modulus between 0.7 and 4.0 kPa, these models succeed in mimicking soft tissue stiffness and the suitability to model hepatocellular carcinoma [44]. Therefore, by modulating the material stiffness properties of the hepatocytes vary. Polyurethane is also emerging as a suitable material for culturing cancer cells but upon fibronectin coating modification which allows cells to attach and proliferate [45]. Polyurethane coated with fibronectin to enable cellular attachment and proliferation is an emerging material for culturing cancer cells. Similarly, hybrid hydrogels prepared from chitosan and poly (γ-glutamic acid) have shown promising results for pancreatic cancer studies [46].

In addition to being biocompatible, these hybrid polymeric materials can produce scaffolds of controlled and highly interconnected micro-porosity and abnormally long degradation rates [47]. These properties have enabled recently culturing oral squamous carcinoma cells during 3 weeks, without significant changes in the scaffold, establishing the link between the size of the tumor with the production of specific angiogenic factors [48]. Additionally, cells cultured in these 3D systems generally possess higher invasion rates than those cultured in 2D and are characterized by the expression of specific epithelial and mesenchymal markers. The stable sponge-like environment enables cancer cells to grow and develop, recreating an in vivo-like atmosphere and promoting clusters with physiological morphology and chemokine secretion.

6.2.4 Tumor and Drug Response

Tumor response to drugs has classically been studied by investigating the molecular mechanism of the cells. These studies are critical in understanding the drug resistance mechanism that the cells develop over time [49]. Apart from cellular processes, the TME also plays a role in developing drug resistance [50]. Cancer cells generally adopt two distinct mechanisms to establish a protective action against drugs. First, an interplay between various interleukins secreted by non-tumor cells support cancer cell survival and block apoptosis. Second, an interaction mediated by the TME upregulates integrins to activate signaling cascade to stop the induced apoptosis.

6.2.4.1 Influence of Interleukins on Drug Response

Interleukins (IL-1,4 and 6) are the pro-inflammatory cytokines that mediate the proliferation of cancer cells and help them escape the defense surveillance of the immune cells [51].

There are two types of IL-1, i.e., IL-1ß and IL-1α In general, IL-1 is tracked in all cancer development stages and is responsible for protecting the cells from therapeutic drugs [52]. This is carried out by the secretion of inflammatory molecules like MMPs, VEGF, chemokines, integrins, etc., which block the drug receptors. IL-4 is an indicator of tumor aggressiveness and invasiveness. Cancer cells produce IL-4 for the upregulation of cathepsin production, which is critical for metastasis, growth, invasion, and angiogenesis [53]. IL-4 also increases drug resistance by expressing CD133 cell surface markers and establishing an anti-apoptotic pathway in the cells, protecting them from chemo radiations [54].

IL-6 is predominantly produced by bone marrow stromal cells and indicates the growth and development of myeloma cells. IL-6 enhances cells to become resistant against the apoptotic stimuli from drugs such as dexamethasone or vitamin D3. Additionally, IL-6 is known to activate the MAPK and JAK/STAT pathways, which are prime in blocking cell-mediated apoptosis in the presence of drugs. This activation results in the regulation of the STAT3 dimerization and production of the anti-apoptotic protein Bcl-xl, enabling cells to avoid chemotherapeutic drugs [55].

6.2.4.2 Cell Adhesion-Dependent Drug Resistance

Cell adhesion-dependent drug resistance (CAM-DR) occurs when the cancer cell interacts with specific receptors of the microenvironment or other cells. These established contacts lead to upregulation of p27*kip*1 and drug resistance. Along with the abovementioned proteins, integrins are the major players in the drug resistance mechanisms of cancer [56]. Integrins participate in the intracellular signal transduction mechanism giving higher survival opportunities to cancer cells. For instance, integrin ß1 and fibronectin cause suppression of apoptosis and are indicative of high cell survival rates against treatment. Furthermore, VLA-4 and VLA-5 specifically have been highlighted as the most critical integrin receptors giving rise to resistance against popular anticancer drugs, such as Doxorubicin or Melphalan. The role of cell-mediated drug resistance comes into play when the integrins and fibronectin bind their respective receptors, leading to the phosphorylation of the Bcl-2 family of protein. This process triggers a cascade of reactions initiated by the PI3 kinase/AKT pathway, which is well known to play a central role in cell survival and drug efflux strategies [57].

6.2.5 Engineered 3D Matrix to Study Tumor-Associated ECM Alterations

Tumoral tissue strongly affects and is in continuous communication with the surrounding ECM. The secreted MMPs and growth factors change the behavior of the ECM, helping the cells proliferate, invade, and metastasize. During the entire

process of tumor development and progression, the matrix keeps on altering. Detailed characterization of the changing ECM has been performed in stand-alone studies, exploring various molecules that make up the matrix. For example, collagen I levels are elevated in primary breast, liver, and lung cancer [58].

Similarly, HA is overexpressed in breast, prostate, colon cancer resulting in poor prognosis and metastasis [59]. Variations of laminin production, where laminin-111 decreases and laminin-332 increases, are common in breast cancer. However, all these factors have not been aggregated into a comprehensive disease model because of the matrix's complexity. As an alternative, genetically modified models such as Cre/Lox are now a popular platform for studying cancer. This section analyses the various engineered models to study cancer progression and the methodologies to recreate the microenvironment [60, 61].

6.2.5.1 Engineered ECM Models

In multicellular organizations, cellular mutations trigger changes in both the cells and their interaction with the environment. The disease progression strongly impacts the cell-matrix biology, a process often studied by mimicking the ECM on a chip using biomaterials. For example, the complex intravasation and extravasation of cancer have been studied on endothelial cells in fibrin gels. The model was later enhanced by incorporating other patterned growth factors and chemokines, enabling a step-by-step controlled replication of tumoral complexity. However, the model was time-limited by the biomaterial. While fibrin is a good model at the early stages of development, as the tumor grows tends to become more fibrous, diverging from fibroin. In later stages, collagen hydrogels provide a more physiologically relevant replica of the ECM, highlighting the importance of choosing the right biomaterial for the study. Additionally, collagen is suitable for patterning structures and topographies, enabling the incorporation of geometrical cues [62, 63]. Lumens of a specific diameter, for example, help alining epithelial cells and produce tubular structures of cancer cells, closely mimicking fallopian tubes and enabling the study of ovarian cancer [64, 65].

6.2.5.2 Biomaterial Approach

To better understand the influence of ECM on tumor progression, the native biomaterials of the ECM have been used to reconstruct the tumor microenvironment. This reconstruction of the ECM in artificial scaffolds is accomplished either using the whole ECM (e.g., decellularized matrix) or individual ECM components [66]. Decellularized scaffolds are extracted from native tissue and considered to retain the chemical cues required for cell function. In the case of cancerous tissue, proper decellularization of the ECM has been demonstrated to retain factors leading to high chances of developing cancer. Additionally, the patient-derived tumor-affected ECM shows increased migration of cells when compared to the healthy decellularized ECM [67]. Similarly, spheroids made from colon cancer grow faster on cancer-derived ECM when compared to a healthy ECM. Similarly, spheroids made from colon cancer grow faster on cancer-derived ECM compared to a healthy ECM [68]. While decellularized cancer-derived ECM is one of the best platforms to

study cancer progression and probably the closest possible composition of the tissue matrix, sourcing, preparation, and consistency are of extreme complexity. A more reliable solution (at the expense of significance) is the fabrication of scaffolds from the matrix protein. Gels based on Collagen I from explants are primarily used to produce breast cancer models. Recently, these scaffolds have gained popularity as platforms to add other ECM components [69]. Similarly, native fibronectin and collagen I combinations are popular models to study breast cancer invasion, migration, and MMP production [70].

6.3 Tumor on a Chip

Tumor-on-a-chip models have soared in the last decade because of the inflection point they represent in the field of in vitro experimentation. Tumor-on-a-chip are: (1) relatable to in vivo 3D tissue, (2) enabled for dynamic studies because of their microfluidic component, (3) easy to monitor compared to animal and patient models [71].

Conventional platforms have set credible benchmarks to study and understand the biology and mechanism the cancer cell plays in the presence of various cues. However, their 2D design (i.e., monolayer) strongly differs from the three-dimensional physiological tissue. While 2D systems have been the base of modern cancer research since its inception, nowadays, their limitations have set a glass ceiling in the field, limiting the development of models to carry out pharmaceutical and industrial-level drug testing. Those limitations are triggering a fast transition to standardized 3D tumor models, enabling the acquisition of the relevant information unreachable by 2D setups. In this transition, tumor-on-a-chip models have emerged as promising platforms to mimic the ECM in a controlled fashion to study cancer progression, including the complexity and dynamics of tumor niches [72].

6.3.1 Microfluidic Platform to Model Cancer

Organ-on-a-chip setups are microfluidic devices fabricated from glass, rigid plastics, or flexible polymeric materials, such as PDMS, to culture cells in micro-channels replicating some of the physiological and pathophysiological complexity of an in vivo system. They are named “chips” because, in the absence of custom methods, the initial fabrication processes were borrowed from the micro-manufacturing field (Fig. 6.2).

6.3.1.1 Organ on Chip Technology

The central and common element to most organs-on-a-chip is one or several channels, populated with various cell types, and designed to replicate the complexity of a tissue. These channels are coated or incorporate a biomaterial mimicking the ECM. Additionally, the “chip” configuration allows a dynamic manipulation of parameters such as mechanical stiffness, chemical cues, and cell density

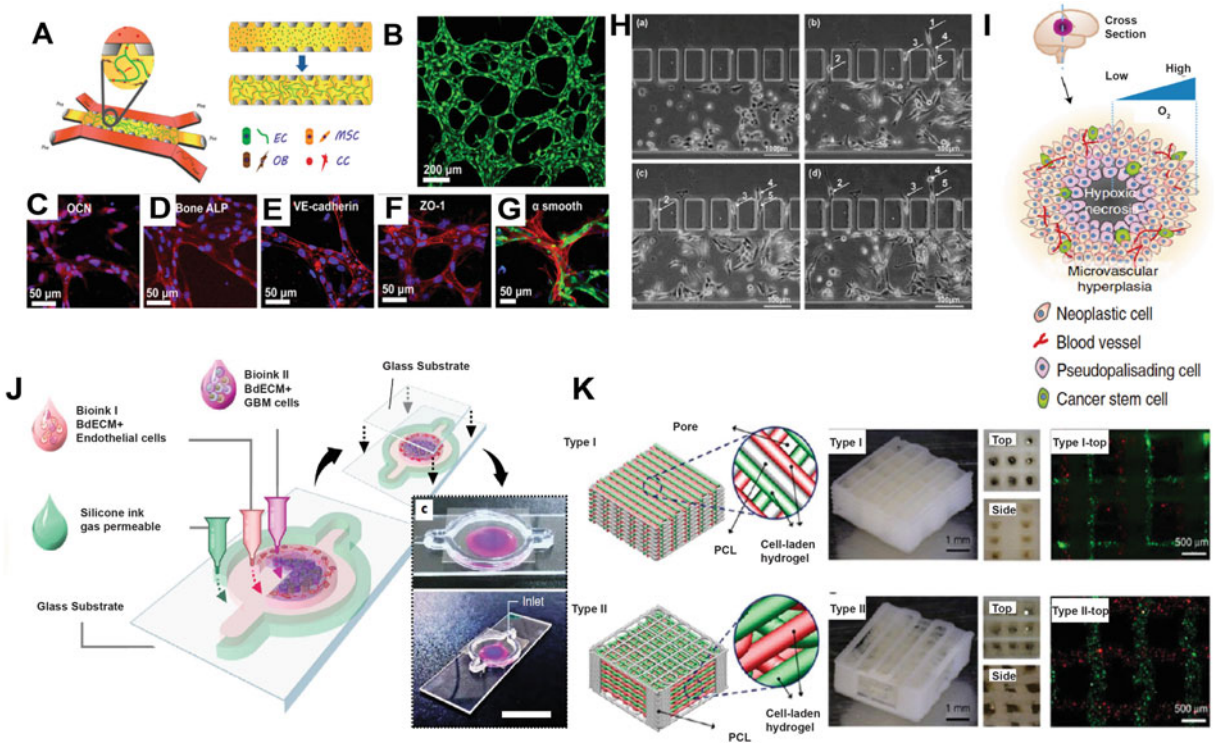


Fig. 6.2 Fabrication of microfluidic devices and adopting 3D printing technology to mimic cancer model. (**A**) Overall depiction of the microfluidic channel used to study breast cell extravasation. (**B**) Microvasculature formed by using endothelial cells in the microfluidic channel as shown in green as they were transfected with GFP. (**C**–**G**) Staining the fabricated microenvironment with osteocalcin, alkaline phosphatase, VE-cadherin, and smooth muscle cells which are secreted by the cells. The cancer cells extravasate through this formed microenvironment. (**H**) Microfluidic device designed to study the migration of breast

Fig. 6.2 (continued) cancer cell (MDA-MB-435S) as seen in the figure. (**I**, **J**) Using bioprinting strategies to print TME. Silicone ink is used to print the boundary, and the cells are encapsulated in the bioink derived from decellularized pig tissue. This model is also validated by computer simulations and to understand the response of the diseased cells to chemotherapy. (**K**) 3D bioprinted model using collagen hydrogel to study the cancer cell migration in a highly vascularized system (Reproduced with permission from [73–76])

[77]. These characteristics enable an unprecedented characterization of the cell-matrix interactions in both diseased and normal conditions [78].

The segregated design of organs-on-a-chip platforms enables multicellular architectures, a feature unachievable in 2D culture platforms. Furthermore, cell viability is maintained for an extended time in these architectures without disrupting the population, thanks to the constant circulation of nutrients and gases. Recent advances have shown the establishment of various organs on chip format and their use to perform tests on drugs, toxins, inflammatory cytokines, or radiation. These complex analyses include mapping the cancer cascade, enabled by framing up the disease in a chip format [9].

6.3.1.2 Growth and Neovasculature

The growth of the tumor cells is vastly regulated by their interaction with the surrounding healthy cells. Breast carcinoma cells T47D, for example, when cultured with human mammary fibroblast cells (HMFs) with various proteins forming the ECM, change their growing pattern to form clusters. Included in a cancer-on-a-chip configuration, the MMPs involved in this change and the influence of the ECM composition were mapped. The precise control of the media enabled by the microfluidic platform enabled the identification of the downregulation of estrogen receptor-α in cancer, which is now exploited to develop hormonal therapies to treat cancer [79].

The freedom to incorporate various cell types, enabled by organ-on-a-chip platforms, has helped establish models addressing the role of cytokines and other paracrine signaling processes in cancer growth, beyond breast cancer. For example, lung cancer cells follow very distinct development patterns when they grow alone or in the presence of healthy lung cells. In the former case, cancer cells fail to proliferate in standard conditions. This result set the ground for finding the specific growth factors secreted by the healthy cell that are prime for the tumor cells to survive and develop.

As mentioned above, the survival, proliferation, and extravasation of cancer cells—and their progression from hyperplasia to neoplasia—are strongly linked to the neovascularization of the tumor site [80]. This critical role has motivated the development of several microfluidic platforms recreating the micro-vessels and capillary sprouting design of tumoral vasculature [81]. Due to their hollow architecture, sacrificial 3D printing is one of the preferred methods to design and produce micro-channels. These structures are transformed into hollow structures, suitable to harbor endothelial cells, by the solubilization of the sacrificial material [82]. The monolithic close design enables continuous media perfusion with leakage, closely mimicking the design of blood vessels [83]. These platforms have enabled, among others, the demonstration of a consistent cell sprouting from the vessel in response to the perfusion of tumor angiogenic factors. Furthermore, the suitability of these systems to rapidly compare cellular responses to changes in the media has enabled the evaluation of those results in the presence of a library of various growth factors and concentrations (e.g., VEGF, HGF, and bFGF).

Another popular design for microfluidic devices modelling vasculogenesis is the fabrication of ECM gel-filled chambers containing endothelial cells within a cell-free environment. Because of their relative simplicity, these models are broadly used to study interactions between cancer and endothelial cells in varied conditions [84].

6.3.2 3D Printing Cancer Models

Because of the extraordinary complexity of cancer and its metastasis process, their interpretation using 2D and animal models is cumbersome, expensive, and prone to fail to capture the critical aspect of disease progression. This lack of suitable models is resulting in a decreasing efficiency of the drug discovery process. Animal models permit systematic investigation for understanding cancer progressing. However, due to new ethical concerns and regulatory policies, the use of animals is nowadays the last resource, rather than the traditional primary approach to study processes such as ECM-cell interactions, cell proliferation, and migration.

In the last decade, the field of physiological models has branched out towards the advancement in tissue engineering, aiming to substitute animal models with equally relevant and less unethical options. This approach is giving a significant boost to the replication of the matrix-cell paradigm, closely studying the mechano-chemical properties of the cancer cells with the help of biomaterials [85]. These biomaterials can mimic the organization of the ECM and precisely recapitulate the tumor microenvironment [86]. When incorporated in bioreactors, they overcome the limitations of current models reproducing physiological conditions, emerging as strong candidates to revolutionize medical cancer research [87].

3D printing has advanced to the levels of minutely incorporating polymer and cells to fabricate a replica of an organ of interest with proper nutrient and oxygen diffusion, provided by micro-channels printed simultaneously during the manufacturing process [88]. The entire systematic process of designing, fabricating, and modelling tumors with 3D bioprinters is very detailed, and the hopes for leading future drug innovation are very high [89].

In 3D bioprinting, the traditional extrusion-based technique gives control over porosity and produces mechanically robust constructs [90]. However, direct and inkjet printing are better fitted for incorporating cells while printing with a reasonably defined resolution [91, 92]. These techniques allow the usage of a broader range of cell-friendly polymers and can be incorporated into the ink while printing. Recent advances in using decellularized scaffold-derived inks have brought together a heterogenous protein composition and intricate geometry similar to the tumor microenvironment [93].

The manufacturability of the bio-inks can be enhanced by chemical modifications, becoming photocurable materials suitable for more complicated architectures [94]. This enhanced manufacturability has enabled, for example, the production of 3D spheroids with MCF-7 breast cancer cells with a hypoxic core using gelation and PEGDMA hydrogels [95]. Similar results have been produced with the direct extrusion of PEG-based hydrogels, which are employed to create

microstructures enabling the formation and long-term culture of cancerous spheroids. Later on, these spheroids are used to investigate drug resistance and malignancy hallmarks in the tumor cells, providing new insights into the proteins secreted in hypoxic conditions enabling drug resistance. This mechanism, for example, is absent in 2D platforms.

3D printing enables printing intricate and defined structures, such as vasculatures, comparable to those in vivo. However, in addition to producing complex geometries, 3D printing also enables tweaking the micro-architectures and stiffness of the matrix and their influence on cell migration and proliferation [96]. This versatility opens a new venue to study crucial parameters in understanding cancer cell behavior that are often overlooked on 2D systems, such as cell displacement, velocity, and path straightness.

The geometrical and mechanical advantages of bioprinting add to the versatility of the cell population. This versatility is apparent in co-culture studies, such as the recent printing of OVCAR-5 ovarian cancer cells with MRC-5 fibroblasts on Matrigel, to understand the synergies between both to promote proliferation and migration [97]. The model also incorporated drug candidates to study their efficacy to prevent tumor progression. Similar studies using fused-filament 3D printing have been performed to study the influence of a bone-like microenvironment in the proliferation and migration of breast cancer.

While most studies in 3D printed models performed to date have been conducted using immortalized cell lines, the field is rapidly moving toward the use of patient-derived cells. This transition is a significant step toward drug discovery and critical in the development of personalized medicine.

6.3.3 Towards Personalized Medicine

The traditional "one-size-fits-all" idea of medicine is rapidly adapting to incorporate the heterogeneity and variability of human biology [98]. Each individual's biology is different, even the biology of one individual at different time points changes. However, the precision achieved in many medical procedures enables their accommodation to this conventionally overlooked variability. The type of drug used in cancer treatment is as critical in its performance as the procedure and dose employed. However, the correct dose is a moving target, depending on the patient's metabolism and situation, and usually aimed through a trial and error process. The new generation of medical procedures aims to diversify the process of drug discovery and utilization. Instead of using a generic model, perform the development process focused on the patient's specific biology. The main barriers to achieving personalized medicine's ambitious and logical goals are the cost, sample preparation, and delivery system required for such development [99]. Moving the medicine out of the patient to overcome these barriers requires the development of biomaterials and devices designed and fabricated customized for patient-specific analyses.

6.3.3.1 Device Fabrication Based on Patient Information

Mass manufacturing is based on the parallel replication of a single process, enabling a theoretically infinite scaling of the process and its efficiency [100]. The same approach is also the base for fabricating and using biomaterial-based devices for patient-specific medicine. The idea is to fabricate a series of devices with physiological relevance for a particular patient and disease. Therefore, these devices have a set (but modifiable) biological, chemical, and mechanical configuration, enabling the study of the effect of a specific treatment. Furthermore, by parallelizing different conditions and treatments, such devices aim to identify the best approach for the case. Technologies such as additive manufacturing, photolithography, and direct ink writing are the main in the device manufacturing process for their combination of versatility and high throughput [101]. Combined with the recent advancements in material chemistry, they are being used to produce devices focused on recording and analyzing patient-specific data in real-time. Biopsy tumor testing kits offer a different approach to producing patient-specific information but are hampered by labor-intense procedures [102]. They use immunohistochemistry (IHC) or in situ hybridization (ISH) to identify the relevant genes involved in developing a specific tumor. In order to access similar information but without lengthy laboratory procedures, the method is being substituted by a combination of qPCR and microfluidic devices. The microfluidic platforms are designed to recognize the exact gene mutation (e.g., Her2 or KRAS) and, therefore, provide valuable information to narrow down possible drugs for the treatment [103]. These microfluidic platforms are coated with various types of matrix proteins to enhance the quality of the data acquired [104]. The cell-friendly surface enhances the integration of the cells from the biopsies, resulting in better characterizations and, ultimately, higher quality data, suitable for machine-aided algorithms and computational analysis. Additionally, the data can also be used in reverse to identify patients that would require treatment. Patient-specific circulating cancer cells, which often branch out from the primary cancer site into the bloodstream, have also been extracted in microfluidic devices to formulate drugs and design therapies. The most well-established system using this approach isolates CTCs escaping from breast, colorectal, and prostate cancer [105]. It utilizes the EpCAM markers expressed by epithelial cells to separate the CTCs from the remaining cell population.

6.3.3.2 Enhanced Drug Delivery to Tumor

Drugs administered into a living host will immediately be coated by various biomolecules, predominately proteins, significantly affecting their activity [106]. Critical parameters such as stability, size, and adsorption get impacted, significantly modifying the original pharmacokinetics [107, 108]. This modification can happen in any direction. For instance, when the nanoparticle-loaded drugs are covered with albumin or apolipoprotein, their activity is greatly reduced, but their encapsulation by plasma membrane proteins will enhance it [109]. Therefore, the right drug may be unusual if it cannot perform in this environment. Because of this, the nanoparticles used as drug carriers with physicochemical properties suitable to adapt to these varying conditions are critical to drug delivery.

The relation between blood circulation and drug circulation half-life is as crucial as drug adaptation to encapsulating proteins [110]. A significant downside of this approach is the encapsulation of the drugs with surrounding proteins. The functionalization of the particles to promote their hydrophilicity has traditionally been used to improve the process, and recent technologies, such as pegylation of the carrier, have shown enrichment of the protein clustering and an associated non-specific macrophage drug uptake [111]. Similarly, modifications of the nanoparticles with proteins targeting the inhibition of phagocytosis (e.g., CD47) are also producing positive results [112].

After the delivery profile and the drug half-life, drug penetration into the tumor site is the last crucial controllable aspect defining the outcome of a drug. The diffusion kinetics of a drug is a consequence of its size and binding affinity [113]. Molecules with high binding affinity are characterized by short penetration distances, while those characterized by weaker interactions with the tissue are prone to traverse it. Similarly, large drugs and drug carriers, independently of their nature, are more susceptible to entangle in the tumor microenvironment than their small counterpart. As a result, fewer amounts extravasate to the target site [114].

The use of drug carriers enables and independent control of such parameters without affecting the chemistry of the drug. For example, drug carriers of about 15 nm and a surface functionalized with cyclic peptides are considered ideal for rapid tumor penetration. Similarly, 10 nm quantum dots loaded in 100 nm spherical nanoparticles are becoming a standard for rapidly reaching a tumor and diffusing a drug [115].

6.3.3.3 Challenges in Clinical Translation

The rapid emergence of novel techniques to design and fabricate tools in the last two decades has enabled a significant leap in the development of therapeutics and the understanding of the environmental factors playing a role in cancer cell progression and extravasation. However, the large-scale screening of therapies is still hindered by suboptimal reproducibility and precision [116]. The current strategy to improve the screening efficiency and make it high throughput goes through the substitution of traditional 2D cultures by 3D organoid culture systems. Forming embryoid bodies can be routinely done by various methods (e.g., digital manufacturing and microfluidic platforms), enabling the upscaling of the testing pipeline and enhancing its physiological relevance [117]. In addition to the incorporation of patient-specific samples, this efficiency is a critical step to generalize the production of customized treatments, which requires studies on a genetic level, running numerous trials, and low-profit margin.

6.3.4 Contribution of AI in Cancer Biology

Years of documented medical cancer research have produced outstanding amounts of data. This data is not only abundant but also unstructured and, in many cases, contradictory due to the disease's dynamic nature. Although this knowledge banked

through years of research has made enormous contributions to advancing medical treatments, its complexity prevents its efficient use by physicians to predict prognosis accurately. Machine learning has emerged as a promising tool to overcome human analytical limitations in cancer research [118]. These algorithms are discovering unobvious patterns and finding out relations between the disease onset and its future development. Therefore, machine learning complements the new physical tools and devices for personalized medicine, providing the tools to handle the unprecedented amount of data collected.

6.3.4.1 Machine Learning in Cancer Prediction and Survival

Machine learning algorithms predict the prognosis of the disease by mapping it, using modelling tools based on supervised and unsupervised learning [119]. These tools can identify and isolate unobvious parameters related to cancer susceptibility, survival, and relapse rate. Beyond predictions associated with cancer properties, machine learning algorithms have proven useful linking phenomena at very different scales, such as the properties of a specific tumor and its diagnosis [120]. Deep learning tools can infer, from biopsies, patient-specific information such as life expectancy, survivability, and treatment sensitivity [121].

Similarly, due to the human analytical limits and time availability of physicians, the usual diagnosis is based on histological and clinical data [122]. It rarely covers in-depth features like family background, diet, or habits that play a role in predicting cancer development. With swift improvement in analytical techniques, such as microscopy, physicians now have access to much more valuable data, yet its greater complexity results in even more challenging predictions. Therefore, machine learning tools are becoming increasingly popular tools for physicians to predict disease progression [123].

6.3.4.2 Clinical Application of the Prediction

The use of machine learning in health care was nominal only 10 years ago [124]. Since then, it has rapidly developed, being ubiquitous in imaging platforms and analytical tools, revolutionizing the treatment paradigm. In radiology, machine learning gives radiologists an upper hand by providing them with tokens of digital knowledge, which adds to the clinical data they already have access to [125]. Incorporating artificial intelligence into medicine does not aim to replace radiologists but rather help them use amounts of data that would otherwise be unmanageable. The algorithms help run analyses with great accuracy and precision, helping, for example, enhance images or generate results useful in the treatment process.

In oncology, the heterogeneity of the tumor requires analyses based on cells at the individual, or close to the individual, level. The genomic and proteomic data, necessary to produce custom and personalized drugs, occur at that level. However, the vast and complex data generated by next generation sequencing makes the analysis unreachable without the use of artificial intelligence algorithms. Therefore, with a future medicine based on genetic information, artificial intelligence plays a critical role in the future of therapeutics [126, 127].

6.4 Cancer Vaccines

Cancer disease most probably is the therapeutic medical field that has advanced the most since its inception. Its parallel progression improving technology, specificity, deep learning methods for diagnostics and radiology, specific target selection, and developing checkpoint inhibitors are unmatched. The field's focus on eradicating cancer is equally absolute and ambitious and has triggered the development of new fields focused on that goal. Cancer vaccines, for example, aim to induce tumor regression with zero relapse tendency, eliminate the adverse effect of drugs, and produce memory cells preventing any relapse.

6.4.1 Types of Cancer Vaccines

Cancer vaccines aim to create a specific response to tumor antigens. Tumor antigens are proteins overexpressed in cancer and playing a role in its initiation and progression [128]. Therefore, the choice of antigen is the single most crucial component of cancer vaccine design. Since protein production is ubiquitous in normal and diseased cells, the challenge is to isolate those produced by cancer cells for survival and not by normal cells (Fig. 6.3).

6.4.1.1 Virus Based

Our body has evolved complex and efficient mechanisms to respond against viruses, and various viruses are studied as candidates for a cancer vaccine making use of those mechanisms. When a viral pathogen invades our immune system, it causes the activation of antigen-presenting cells (APCs), which is triggered by the interaction of the viral antigens with pattern recognition receptors [133]. Exploiting these interactions, viral vaccine vectors, such as poxviruses and adenoviruses, have been used for therapeutic applications in cancer.

The main drawback of using virus antigens to tag cancer cells is the neutralization effect of the viral vectors by the body, difficulting the use of booster doses. However, this limitation can be circumvented in some cases. For example, PROSTVAC-VF/Tricom, a virus-based vaccine for prostate cancer, is designed to dodge the immune system with booster shots containing the same tumor antigen but different viral vector or vector type [134]. Additionally, the activity of the vaccine can be enhanced with virus-based immunotherapy regimens, which are primed with checkpoint inhibitors—this therapy with prostate-specific membrane antigen is under clinical trials. The therapy is further enhanced by coupling it with subcutaneous injections of antibodies, such as CTLA-4 antagonist or tremelimumab [135].

6.4.1.2 Tumor Based

Vaccines carrying either killed cancer cells or patient-specific antigen-presenting cells (APCs) with cancer antigens are developed to have specificity against the disease and are irradiated to restrict further cell division [136]. Some of these vaccines, for example, are prepared using whole tumors explants that are genetically

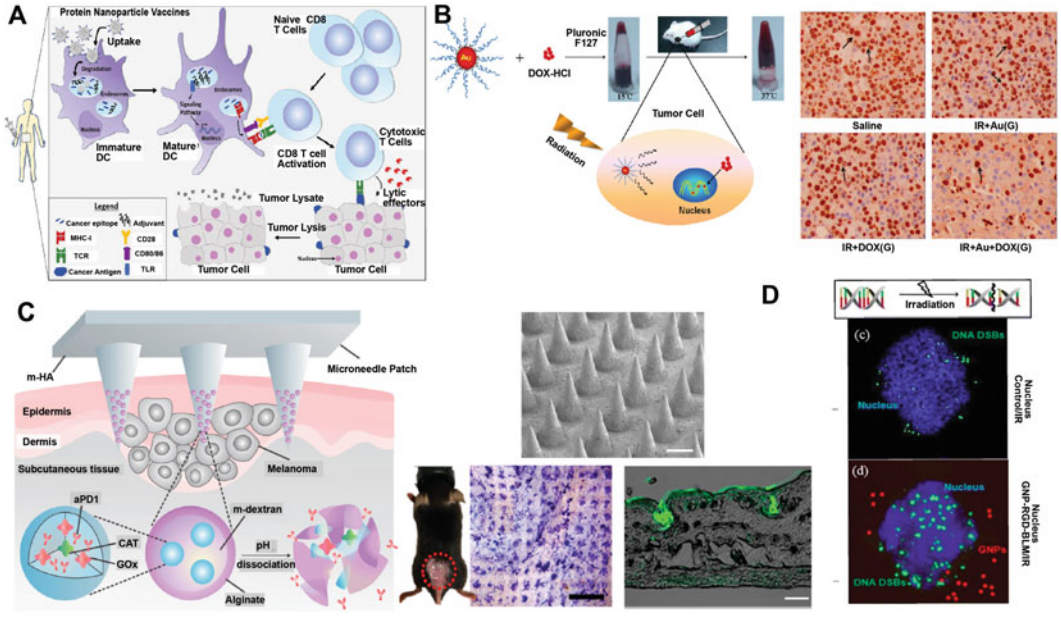


Fig. 6.3 Cancer therapeutics in using biomaterials as drug vehicle. (**A**) Depiction of cancer elimination from the system by using protein nanoparticles as cancer vaccine. (**B**) Combinational therapy using both nanoparticles and radiation therapy. Pluronic hydrogel is loaded with both gold nanoparticles and Doxorubicin, followed by exposure to IR radiation. As seen from the Ki-67 immunohistochemistry, the panel with radiation gold nanoparticles and Doxorubicin has higher effectiveness compared to the control sample with saline treatment. (**C**) Dextran nanoparticles used in microneedles to deliver drugs for melanoma treatment. The image shows the penetration of the microneedle with drugs into the mouse skin. The green signal shows FITC positive penetrated antibody loaded microneedle. (**D**) Cancer therapy combining gold nanoparticles with radiation therapy. As seen from the image, the cancer is highly targeted, causing DNA damage when gold nanoparticles are accompanied by IR radiation. (Reproduced with permission from [129–132])

modified to secrete cytokines and granulocyte–macrophage colony-stimulating factors. These GM-CSF play a significant role in antigen presentation and survival of dendritic cells [137]. Vaccines working on this principle have shown potent action on murine models but a low success rate in humans. However, those successful cases, such as Sipuleucel-T, which is FDA approved for prostate cancer, are hampered by costs preventing worldwide use [138].

Other cell-based vaccine preparations using bacteria and yeast have succeeded in stimulating an immune response. This approach was first demonstrated by Coley et al. using heat attenuated bacteria to treat cancer patients. Since then, many other examples have emerged [139]. For example, BCG (also heat-inactivated) is commonly used to treat bladder cancer. Other [140] species of bacteria, such as attenuated strains of Salmonella and Listeria, have also been used as cancer vaccines. These are internalized after infection and help the APCs deliver DNA-or RNA-encoded tumor antigens, followed by a robust antitumor immunity.

6.4.1.3 Peptide-Based

Peptide vaccines are based on a single antigen-based short peptide showing an immune response, potent enough to be employed for therapeutics [141]. Existing examples use short peptides and a single antigen, neglecting the heterogeneity of the pool of antigens, and are characterized by reduced immune responses [142]. This reduced response arises from the tendency of small peptide moieties to directly bind MHC class I molecules, bypassing the APCs processing and increasing T-cell dysfunction and tolerogenic signal. Efforts to improve the design of the vaccine focus on enhancing the activation of T-cell responses by incorporating peptides into constructs that are either amphiphilic or combined with inflammatory and immune modulators. Poly-IC and Poly-ICLC are ligands that have succeeded as adjuvants in peptide vaccines in experimental setups [143]. Additionally, some long peptides containing both MHC class I and II moieties have succeeded in eliciting CD8 and CD4 T-cell response through DC cell processing rather than APCs [144]. These long peptides produce a more robust T-cell response than short peptides, with the additional advantage of a simultaneous induction of memory T-cell response [145].

6.4.2 Biomaterials for Cancer Immunotherapy

Biomaterials are a necessary piece to study cancer progression by recapitulating its native microenvironment in vitro. In the last decade, the field has developed from the original goals focused on biocompatibility and survival of an encapsulated cell population to reproduce specific tissue properties [146]. This growing interlink between materials and biology promotes a new paradigm, where materials move beyond their support role to become essential in controlling cellular pathways [147]. This new paradigm has profound implications on the role of biomaterials in cancer drug testing and therapeutics.

6.4.2.1 Drug Delivery

Current strategies to fight the progressing disease of cancer are a combination of surgical interventions, chemotherapy, and radiation therapy. The main downside of these methods is their suboptimal specificity. They effectively block DNA synthesis and mitosis in cancer cells but also affect the functionality of healthy cells [148].

The collateral damage of cancer drugs partially results from the high doses required due to their poor accessibility to the cancer cells [149]. Therefore, there is an immediate need for therapies that actively or passively target the cancer cells only, avoiding the need to flood the system for a significant amount to reach the cancer site. Towards that objective, several materials have been developed with the specific aim to deliver drugs directly to the cancer site—synthetic and natural biopolymers, lipids, hydrogels, and nano-inorganic carriers, to name but a few. Furthermore, these carriers are in continuous enhancement by other fields, as they are designed to easily bind to ligands like DNA, RNA, and proteins, simplifying the process of drug delivery [150, 156].

Inorganic carriers (generally based on metal cores) are bringing new functionalities to the field. Quantum dots, for example, have already proven to be very effective in both drug delivery and tissue imaging. Because of its size (1–10 nm), large surface-area-to-volume ratio, photoluminescence, and uniformity, QDs have emerged as one of the best methods [164]. Their main drawback is their intrinsic hydrophobicity, resulting in a tendency to form aggregates [158]. Nevertheless, coating the QDs has proven to be an effective strategy to increase their affinity to water and bioactivity. Similarly, carbon nanotubes (CNTs) have recently found a unique application as the central element in photoablation therapy, where they transform incident light into heat, selectively raising the temperature of tumoral tissue [151, 155].

Superparamagnetic iron oxide nanoparticles (SPIONs) are another inorganic carrier that is now increasingly being used. They are characterized by magnetism, easy visualization, and biocompatibility, playing a central role in modern magnetic resonance imaging [152].

Organic carriers based on nano-size biocompatible polymers complement have also found direct application in cancer treatment and diagnostic. These polymeric vehicles can be prepared from synthetic polymers such as PCL, PLA, HPMA, and PLGA or natural polymers like chitosan, gelatin, and collagen [157, 160]. Unlike their organic counterparts, drugs encapsulated in these bioabsorbable polymer matrices are released by erosion or swelling, followed by diffusion. This process, significantly slower in synthetic polymers than in natural polymers, provides the former with the advantage of a prolonged drug release, which can be sustained for several weeks. For example, Doxorubicin is a drug used to treat solid cancer and typically encapsulated in a hydrogel by conjugating it to a sugar moiety (i.e., dextran) [165]. Similarly, PLGA has been used to embed Tamoxifen, an anticancer drug cleaving tumoral DNA [161]. Carrier and cargo are designed as part of a unique system rather than as separate parts in all cases. In the design process, factors such as the drug-to-polymer ratio, the polymer's molecular weight, and the polymer's source affect the drug's delivery and effectiveness [153].

Liposomes, lipid bilayers with enclosed aqueous centers, are an emerging carrier for hydrophilic drugs. They have unparallel biocompatibility and effectiveness but suffer from difficult clearance from the system. Midway between the polymer and the liposomes are the hydrogels, characterized by a very porous core and unmatched ability to absorb biological fluids. Hydrogels are primarily used to encapsulate hydrophilic drugs [159]. However, their susceptibility to modifications makes them suitable to carry other drugs and perform controlled drug release, targeted drug delivery, and promote bio-adhesion.

6.4.2.2 Combination Therapies

While some patients in advanced stages of cancer tend to respond more favorably to a single treatment, in the vast majority of clinical cases, combination therapeutics show higher effectiveness. For example, a combination of regulators expressed by T-lymphocytes, such as CTLA-4 and PD1(Nivolumab), results in a synergetic effect where CTLA-4 (Ipilimumab) plays a role in dampening the T-cell priming, and PD1 blocks the effector response of T-cells [141]. The efficacy of the treatment increases by 60% when the drugs are used in combination with respect to their use in isolation. However, this increased efficacy comes at the cost of additional toxicity to non-cancerous cells, comparable to autoimmune diseases.

Combining chemotherapy and checkpoint inhibitors is also a usual strategy to lower the impact of tumor treatment in other cells. For instance, Cyclophosphamide is known to deplete T regulatory cells, and when combined with chemotherapeutic drugs like Paclitaxel, it enhances antitumor T-cell functions and initiates antitumor responses [154, 162]. However, not all combinations of drugs result in a positive effect. Several drugs have shown detrimental effects that largely overcome the possible benefits and have resulted in their discontinuation. As a result of these extreme side effects of chemotherapy and the massive damage that can cause to healthy cells, there is a growing field of research focused on developing molecularly targeted therapies. These therapies are designed to target cells with the specific genetic characteristics of cancer.

Immunostimulatory antibodies are another class of cancer therapeutics that are used as monotherapy or in combination. These are designed to target the TNF receptors in order to activate them. For example, a combination of checkpoint inhibitor anti-CTLA-4 and immunostimulatory antibodies anti-4-1BBL triggers T-cell co-inhibitory blockade in B16 melanoma and prostate tumors [163].

The combination of molecular therapeutics with immunotherapy does not, however, provide optimum results in all situations. For example, while some molecular therapies impart immunomodulatory effects, they inhibit the benefits of the mutations caused by chemotherapy, making immunotherapies less effective. Nevertheless, while current combinatorial strategies are far from perfect, modern studies have demonstrated solid advances in developing effective drug combinations that do not compromise the health of normal cells.

6.5 Summary

Even though tremendous advances have been made in the field to bridge the gap between cancer vaccine development and biomaterials, there is still much work left to translate the research from lab to bedside.

The development of better disease models is critical to remove the disagreement between the immunological response in small animals, large animals, and humans. Furthermore, these models need to be developed within the paradigm of cancer as an individual disease, with particular characteristics and exceptionalities for each patient. In developing biomaterials for cancer study and treatment, it is critical to address the native batch-to-batch variance of the material and the different responses between patients and situations. These two aspects will strongly condition the success of the field upscaling to strategies of general use.

The use of biomaterials as the primary agent for developing potent cancer immunotherapeutic is promising but still has a long path to cover before its application. All the main challenges of the field are, however, characterized by a strong multidisciplinary. Thus, succeeding on the objective of fully understanding and eradicating cancer will ultimately depend on our ability to establish collaborations between scientists from various fields, from medical and material sciences to computer and mechanical sciences.

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