Cancer Letters 380 (2016) 319–329

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Recapitulation of complex transport and action of drugs at the tumor microenvironment using tumor-microenvironment-on-chip

Bumsoo Han [a,b,c,*], Chunjing Qu [d], Kinam Park [b,c], Stephen F. Konieczny [d], Murray Korc [e,f]

[a] School of Mechanical Engineering, Purdue University, West Lafayette, IN, USA
[b] Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, USA
[c] Birck Nanotechnology Center, Purdue University, West Lafayette, IN, USA
[d] Department of Biological Science, Purdue University, West Lafayette, IN, USA
[e] Departments of Medicine, Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[f] Pancreatic Cancer Signature Center, Indiana University Simon Cancer Center, Indianapolis, IN 46202, USA

ARTICLE INFO

Keywords:
Drug transport
Targeted delivery
Stromal tissue
Microfluidics
Nanoparticles

ABSTRACT

Targeted delivery aims to selectively distribute drugs to targeted tumor tissues but not to healthy tissues. This can address many clinical challenges by maximizing the efficacy but minimizing the toxicity of anti-cancer drugs. However, a complex tumor microenvironment poses various barriers hindering the transport of drugs and drug delivery systems. New tumor models that allow for the systematic study of these complex environments are highly desired to provide reliable test beds to develop drug delivery systems for targeted delivery. Recently, research efforts have yielded new *in vitro* tumor models, the so called tumor-microenvironment-on-chip, that recapitulate certain characteristics of the tumor microenvironment. These new models show benefits over other conventional tumor models, and have the potential to accelerate drug discovery and enable precision medicine. However, further research is warranted to overcome their limitations and to properly interpret the data obtained from these models. In this article, key features of the *in vivo* tumor microenvironment that are relevant to drug transport processes for targeted delivery were discussed, and the current status and challenges for developing *in vitro* transport model systems were reviewed.

Introduction

Many promising anti-cancer drug candidates have been identified in the last several decades. However, only a handful have exhibited therapeutic efficacy on human patients. This is largely due to the limited delivery of drugs to target tumors, which can result in unwanted accumulation of compounds to non-targeted healthy tissues and organs, and ultimately lead to systemic toxicity. Targeted delivery, which aims to selectively distribute drugs to targeted tumor tissues but not to healthy tissues, can address many of these difficulties. Such targeted delivery, however, is very difficult to achieve [1]. The term "targeted" used in this article refers to the preferential delivery of drugs to the tumor site. It should be distinguished from "targeted therapy," which refers to drugs interfering with specific molecular targets in cancers.

Recent developments in the field of nanotechnology enables the synthesis of a wide variety of **n**ano**p**articles (NPs), whose size and surface properties can be designed to serve as effective vehicles for targeted delivery. These nanostructures include liposomes, polymer micelles, dendrimers, drug nanocrystals, magnetic nanoparticles, gold nanoparticles/nanoshells, nanorods, nanotubes, and drug–polymer conjugates (all of which will be collectively referred to as NPs). Research aimed at controlling the size and surface properties of these NPs to be responsive to the tumor microenvironment has been performed as reported elsewhere [2–5]. Even though improvements in the delivery efficacy have been shown, the majority of administered NPs fail to reach target tumors. One of the biggest benefits of using NP formulations is to avoid non-aqueous solvents for administering hydrophobic drugs to patients, resulting in fewer side effects, while maintaining the same efficacy. The success of Abraxane® (nanoparticle albumin-bound paclitaxel) and Doxil® (PEGylated liposome formulation), in large part, relies on delivering anticancer drugs without using organic solvents. In order to maximize the therapeutic outcomes, however, drug accumulation as well as penetration into the targeted tumors should be improved. The challenge before us is to achieve effective delivery to the cancer cells since it is significantly hindered by various barriers engendered by the complex **t**umor **m**icro**e**nvironment (TME).

After being administered into a patient's bloodstream, the drugs (for brevity, the term "drug" is used to refer both drug and drug delivery system including NPs) are thought to be subjected to complex and multi-faceted transport processes prior to reaching the cancer

* Corresponding author. Tel.: +1 765 494 5626; fax: +1 765 496 7535.
E-mail address: bumsoo@purdue.edu (B. Han).

http://dx.doi.org/10.1016/j.canlet.2015.12.003
0304-3835/

cells as reviewed previously [6–10]. These include – i) blood flow-driven transport to the tumor vasculature, ii) transvascular transport (i.e., extravasation), iii) interstitial transport, and iv) cellular uptake and metabolism as illustrated in Fig. 1. Excess drugs often occupy the interstitial space or are transported through the lymphatic vessels. These transport phenomena are governed by diffusion and convection processes, and the significance of each process is dependent on both drugs and the biophysical conditions of TME. The drug dependent properties are the size and surface properties, and the TME dependent ones include leakiness of the blood vessel wall, interstitial fluid pressure gradient, and the **e**xtra**c**ellular **m**atrix (ECM) microstructure within the tumor interstitium. These processes and physiological conditions are highly dynamic, interconnected and vary spatiotemporally.

Besides these biophysical barriers, the TME also poses biochemical and biological complexities. Typically, tumor tissues consist of cancerous cells as well as stromal components that consist of various stromal cells including **c**ancer-**a**ssociated **f**ibroblasts (CAF), diverse immune and inflammatory cell types and rich extracellular matrix components, such as type I collagen [11,12]. In addition to the highly heterogeneous cancer cell populations, i.e. intra-tumoral heterogeneity, the complex stromal tissue acts as a repository for various growth factors and cytokines that can dramatically influence tumor growth and drug response. The TME is also a hypoxic environment. Thus, it is important to understand the TME to design and develop effective targeted drug delivery systems. New tumor models that allow for the systematic study of these complex environments are highly desired and will provide reliable test beds to characterize and optimize the design of drugs.

Several tumor models are available, but they do not adequately address this challenge. Conventional static *in vitro* systems, including cell suspensions and cell monolayers, are not sufficient to study these complex *in vivo* transport processes because the model systems lack dynamic interactions among the cells, ECM, interstitial fluid and drugs. Animal models can provide a TME with all of these dynamic interactions, but such models are limited to systematically studying the effects of these dynamic interactions. Recently, research efforts have yielded new *in vitro* tumor models, the so called **t**umor-**m**icroenvironment-**o**n-**c**hip (T-MOC), that recapitulate certain characteristics of the TME. Although various configurations have been developed, T-MOCs are basically microfluidic platforms where cancer cells are cultured within the ECM under perfusion conditions. These new models show benefits over other conventional tumor models, and have the potential to accelerate drug discovery and patient-specific personalized treatment planning. However, the TME is extremely complex and there remain significant limitations to overcome. In this article, key features of the *in vivo* TME that are relevant to drug transport processes for targeted delivery are reviewed, and the current status and challenges for developing transport model systems are discussed.

Tumor microenvironment: a complex and chaotic bed for tumor growth

The tumor microenvironment is a complex and adverse environment for drug transport and action. It comprises a highly heterogeneous mixture of tumor and stromal cells embedded in an extracellular matrix that also includes cytokines, growth factors, inflammatory cells and macrophages. Together, the TME poses multifaceted barriers including biological, chemical and physical hindrances to drug transport and actions. These barriers are highly dynamic and often interconnected. Their interactions and relative significance with respect to drug delivery and therapeutic efficacy vary drastically depending on the cancer type, stage and organs. The current difficulty in developing new anticancer drugs and drug delivery systems partly stems from the lack of a clear understanding of the delicate interplay of these barriers at the TME [13–15]. Thus, instead of providing a generic description on these hindrances, it is more relevant to collectively discuss the interplays that are associated with one type of cancer. Here, our discussion will be focused on pancreatic cancer and its associated TME unless mentioned otherwise.

Pancreatic **d**uctal **a**deno**c**arcinoma (PDAC) is a significant clinical challenge due to its poor prognosis and extremely low (7%) five-year survival rate [16]. Its extensive TME presents many key features relevant to discussing the hindrances and resistance of drug transport and actions. One of the most notable characteristics of PDAC is its marked desmoplasia. The desmoplastic stroma of PDAC is composed of CAFs, various immune and inflammatory cell types and a dense extracellular matrix [11,12], as illustrated in Fig. 1. Moreover, PDAC is poorly vascularized and has extremely high **i**nterstitial **f**luid **p**ressure (IFP) [17–19]. In this complex 3D TME, highly intricate and multifaceted interactions occur among **p**ancreatic **c**ancer **c**ells (PCC), CAFs, **t**umor-**a**ssociated **m**acrophages (TAMs) and other

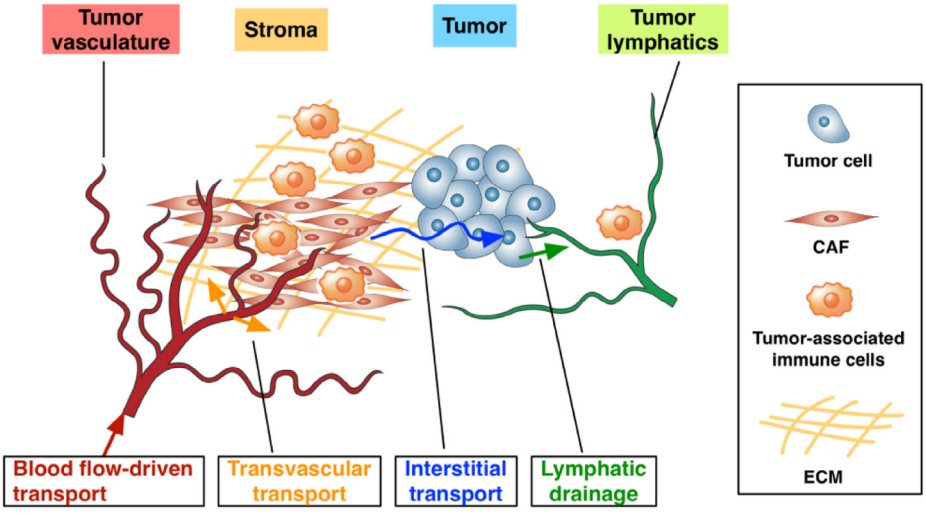

Fig. 1. Complexity of the tumor microenvironment. TME poses multi-faceted barriers to drugs transport owing to the dense stromal tissue, which is composed of collagens, fibronectin, and hyaluronan, an abundance of cancer-associated fibroblasts, and aberrant interactions between infiltrating tumor-associated immune cells, cancer cells, and CAFs.

immune cells. The biochemical and biophysical interactions occurring among the cells within the TME are poorly understood.

PDAC is a complex, heterogeneous and genetically unstable disease which is caused by prolonged accumulation of mutations in a key oncogene and tumor suppressor genes [20,21,22]. These include activation of the *Kras2* oncogene, inactivation of the tumor suppressor genes *Cdkn2A*/*Ink4A*, and finally, inactivation of the tumor suppressor genes *TP53* and *Dpc4*/*Smad4* [23,24]. Recent studies indicate that PCCs carry an average of 63 genetic alterations per cancer, which can be grouped into 12 core signaling pathways [20]. PDAC is thought to arise from precursor lesions such as **pan**creatic **i**ntraepithelial **n**eoplasia (PanIN). These lesions develop into invasive carcinoma through a multistep carcinogenic process (illustrated in Fig. 2).

The complex TME of PDAC poses multiple barriers that inhibit transport and action of drugs. First, the TME serves as a biophysical barrier that impedes effective transport of drugs to target cancer cells or associated stromal cells. After administration, drugs are subject to complex transport processes to reach the cancer cells, including blood flow-driven transport, drug–endothelium interactions, extravasation, interstitial transport and cellular uptake [6,7]. Although the drugs are thought to preferentially extravasate more in tumors than in normal tissues via the so called **e**nhanced **p**ermeation and **r**etention (EPR) effect [27,28], the hypovascularity of PDAC may limit the benefit of EPR effects [29,30]. Nonetheless, a certain percentage of PDACs exhibit a strong angiogenic gene signature and areas of increased microvessel density [31], raising the possibility that drug delivery may be more efficient in this patient subgroup. However, even after the drugs preferentially extravasate into the PDAC TME, the drugs encounter a very dense stroma and significantly elevated IFP. The dense stroma is attributed to activated CAF, inflammatory immune cells and an excessive deposition of a complex ECM that includes dense collagen type I and III bundles, hyaluronic acid, fibronectin and desmin [17,18,32], as illustrated in Fig. 3. The dense ECM microstructure and cell packing of stromal tissue significantly hinder interstitial transport in conjunction with elevated IFP [18,30,33–36]. These features are compounded by hypoxia and infiltration of growth promoting inflammatory cells at the TME.

Second, increasing evidence has shown that various TME components, including the ECM, soluble cytokines, growth factors, the MMP family of proteases, and immunosuppressive and protumorigenic immune cells, contribute to extensive tumor promoting properties. Pancreatic stellate cells are activated into CAFs, which produce and deposit fibronectin and collagens, whereas inflammatory cells and macrophages produce chemokines and cyokines. Thus, the TME of PDAC is rich in growth factors, including **f**ibroblast **g**rowth **f**actors (FGFs), **e**pidermal **g**rowth **f**actor (EGF) receptor ligands, **t**ransforming **g**rowth **f**actor-β (TGF-β) isoforms, and **c**onnective **t**issue **g**rowth **f**actor (CTGF) [19,37,38]. These chemical environments not only facilitate stroma production but also enhance PCC proliferation, **e**pithelial–**m**esenchymal-**t**ransition (EMT), metastatic potential, and importantly, therapeutic resistance through molecular interactions between cancer cells and CAFs. The combination of these complex biochemical environments with hypoxia and heterogeneous genetic mutations makes PDAC incredibly resistant to therapeutics.

The development of **g**enetically **e**ngineered **m**ouse **m**odels (GEMMs) of PDAC has greatly aided fundamental studies of the interactions between PCCs and stromal cells. For example, PDAC CAFs express vitamin D receptors, and activation of these receptors by the calcitriol analogue suppresses deleterious immunological cell infiltrates in mouse PDAC (mPDAC) [39]. Conversely, CAF depletion in mPDAC has been associated with altered immune gene expression and altered infiltrating immune cell populations, including decreased $CD4^+$ effector T cells, increased $CD4^+Foxp3^+$ regulatory T cells (Treg), and decreased cytotoxic $CD8^+$/Treg and $CD3^+$/$CD11b^+$ ratios [40]. *Ctla4* expression was also increased, meaning that treatment of CAF-depleted mice with a CLTA-4 blocking antibody attenuated PDAC progression, improved overall survival, induced tumor clearance in up to 25% of the pancreas, and reprogrammed the transcriptome to a pattern that resembled control (CAF-competent) tumors. Similarly, deletion of *Shh* in cancer cells to suppress mPDAC stroma led to more frequent PanIN and ADM lesions at a young age, an earlier appearance of loss of differentiation of mPDAC, increased metastasis, and more rapid death [40].

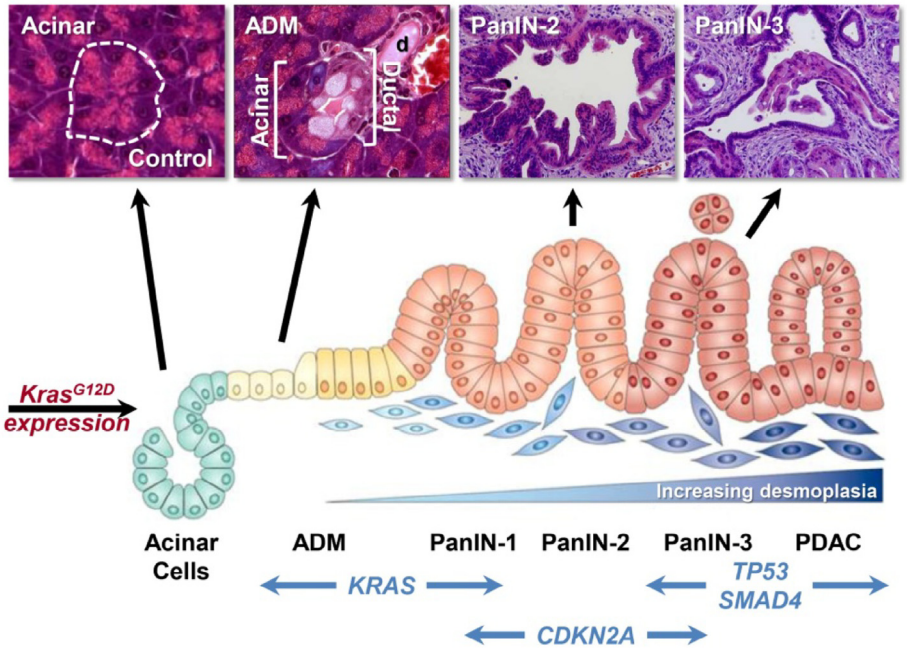

Fig. 2. Schematic model of genetic alterations and histological examples of acinar cells, ADM (acinar–ductal metaplasia), PanIN, and PDAC progression as observed in a mouse model of PDAC. Diagram adapted from Morris et al. [23]. Images from Zhu et al. [25] and Shi et al. [26].

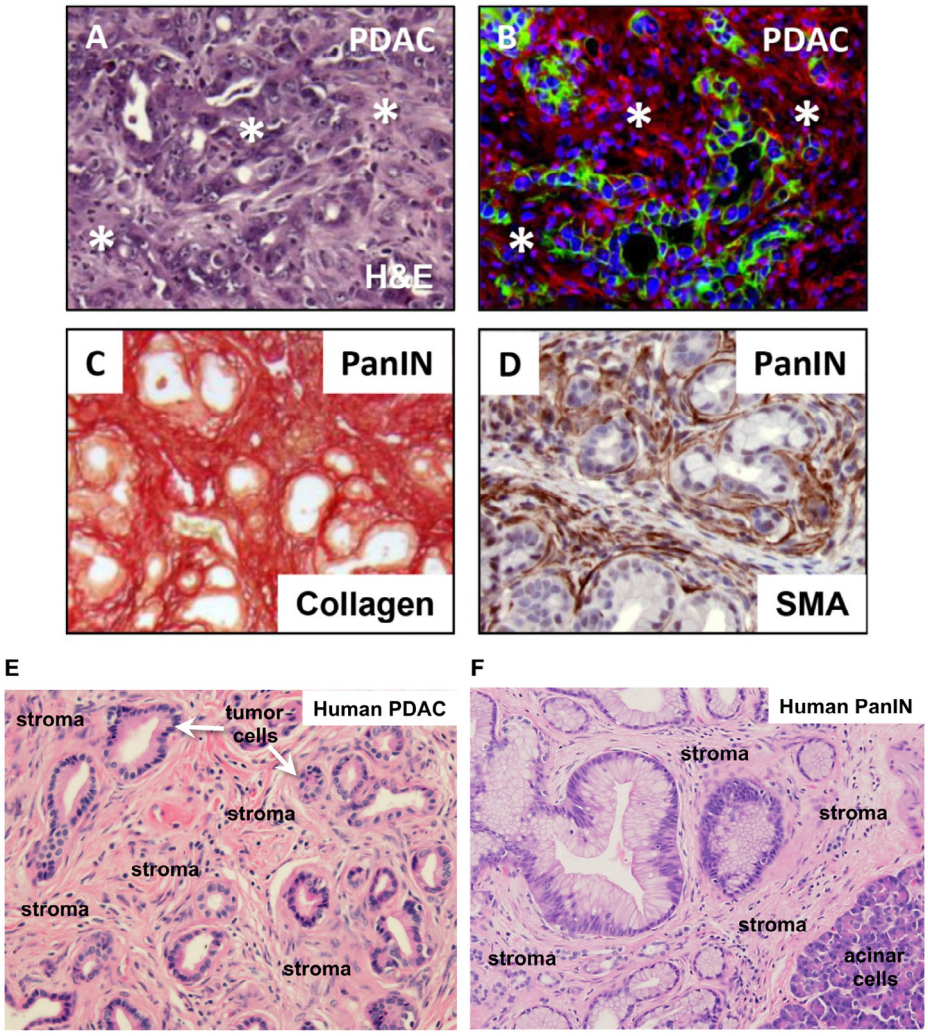

Fig. 3. Stromal components in PDAC and PanIN. (A, B) Mouse PDAC from *elastase-CreER^{T2}/LSL-Kras$^{G12D/+}$/LSL-Tp53$^{R172H/+}$/R26$^{mTmG/+}$* mice. Upon tamoxifen treatment, CreER^{T2} becomes active in the acinar cell compartment, leading to activation of KRAS^{G12D}, TP53^{R172H} and mGFP expression. (B) Membrane localized tdTomato Red (mT) labels stromal cells, whereas membrane localized GFP (mG) marks the tumor epithelial cells. The majority of PDAC tumor mass consists of stromal components (indicated by asterisks in A and shown in red in B). (C, D) Mouse PanIN lesions from *elastase-CreER^{T2}/LSL-Kras$^{G12D/+}$/LSL-Tp53$^{R172H/+}$* mice. Sirius Red staining identifies extensive collagen deposition (C). Similarly, anti-smooth muscle actin (SMA) (brown stain) identifies cancer associated fibroblasts (CAFs) surrounding the transformed PanIN epithelial cells in PDAC samples (D). (E, F) Human PDAC and PanIN H&E stained sections. The predominant stromal components are noted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

There was also enhanced cancer cell proliferation and angiogenesis, increased *Zeb1* and *Slug* expression consistent with EMT, and reduced CD45^{+} myeloid cells and F4/80^{+} monocyte infiltration. Similar results were observed when animals were treated with a Smoothened inhibitor. Genetic *Shh* deletion or pharmacological targeting of *Shh* signaling pathways attenuates stroma formation but leads to more aggressive mPDAC. Thus, depending on the strategy, stroma depletion can cause beneficial or deleterious effects in GEMMS of PDAC.

Transport barriers of TME and drug resistance

Pathophysiological characteristics of tumors present multiple levels of transport barriers to targeted delivery of drugs. These include leaky and chaotic vasculature of the tumor, increased IFP, less functional lymphatic vessels, dense ECM microstructure and high cell packing density [27,28,34–36], as illustrated in Fig. 4A. These TME characteristics are highly dynamic, interconnected and vary spatiotemporally [13,29], and the compounding effects of all of these physiological parameters on drug transport are not yet fully understood. In this section, the complex transport processes of how drugs reach the targeted tumor will be discussed with relevant transport barriers posed by the TME.

Although it is not directly associated with TME, one of the most critical steps of drug transport is blood flow-driven transport. After being administered intravenously, the drugs first circulate in the bloodstream consisting of complex cells and plasma proteins. During blood circulation, a significant portion of the drugs are taken up by the immune cells in the bloodstream including monocytes, leukocytes, and dendritic cells; and in tissues by resident phagocytes, i.e., by the **r**eticulo**e**ndothelial **s**ystem (RES) of the spleen, liver, and lungs [42,43]. This clearance significantly reduces the amount of the drugs available in the bloodstream to reach their intended target. Thus, the surface of many drug delivery systems, particularly NPs, is PEGylated in order to decrease the uptake by the RES and prolong the circulation of the NPs [8,42,44]. Besides the uptake by the immune system, the NPs also interact with other components within the blood [44,45]. These often can result in hemolysis, which refers to red blood cell destruction and degradation of NP integrity. Ultimately, this results in premature release of the encapsulated drugs.

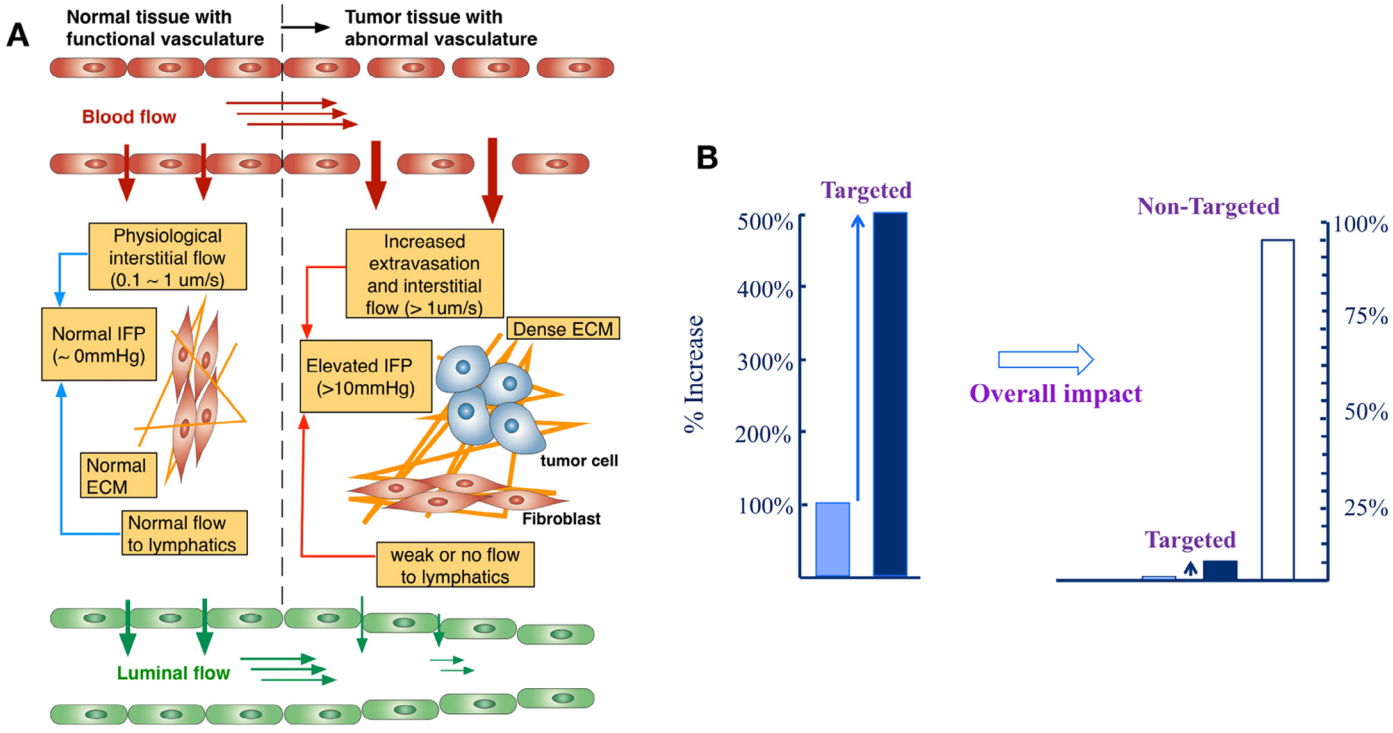

Fig. 4. Transport barriers at the tumor microenvironment, and the outcome of typical targeted delivery. (A) Schematic of vascular and tissue structure relevant to drug transport of normal and tumor tissues. In normal tissue, the endothelium is tightly packed and very low interstitial fluid flow is present. This fluid flows to the lymphatics through the normal ECM, and the IFP minimally builds up. On the contrary, the endothelium of tumor tissue is leaky and has large pores, which leads to high interstitial fluid flow and more extravasation of the NPs. In conjunction with less functional lymphatics and the dense ECM, this increased interstitial fluid flow results in elevated IFP, which adversely affects the extravasation. The compounding effects of the elevated IFP, leaky vasculature, and poor vascularization of the tumor are still not fully understood. Images from Reference 7. (B) Relative NP distribution at a target tumor site. Various NP-mediated drug delivery strategies have been reported to improve the drug accumulation at the intended target, causing up to a 5-fold increase. However, the majority of the administered drug ends up at non-targeted sites. Adapted from Reference 41.

Once the drugs reach the vicinity of their target site after escaping being cleared from the circulation, they are transported across the endothelium of the tumor vasculature to enter the tumor interstitium (i.e., extravasate). The tumor microvasculature is characterized by a highly disorganized network of blood vessels whose endothelium exhibits enlarged intercellular gaps, known as fenestrations, that are heterogeneously distributed across the tumor vasculature [46]. The size of these intercellular gaps, so called vascular pores, for different tumors have been reported to be between 300 and 700 nm, and in rare occasions can be up to 2 μm, which are significantly larger than those found within the normal tissue (typically smaller than 20 nm) [47–49]. These values should be interpreted with caution though, since many of these were measured using xenograft models and very limited information is available for humans. Further research is warranted to validate whether these values are applicable to various cancer types, stages and organs in human patients.

Transvascular transport is thought to be extravasation of drugs by convection driven by the pressure gradient across this porous endothelium and diffusion by the concentration gradient. Thus, the transvascular permeability (i.e., measure of transvascular transport at a given set of pressure and concentration gradient) depends on the size of both the drugs and vascular pores. For small macromolecules with hydrodynamic diameters that are much smaller than the pore size, transvascular permeability is observed to be quite insensitive to the pore size [47]. However, for NPs that are larger in size, e.g. 50–200 nm, the effect of the pore size on transvascular permeability becomes more profound. Transvascular permeability also depends on the developmental state of the cancer. Transvascular permeability was found to be 2-fold greater in primary sites of breast cancer tumors (in the mammary fat) when compared to that at metastatic sites, while the metastatic site was associated with a greater level of vascularization [50]. Yuan et al. [49] experimentally investigated the effect of molecular size on transvascular transport, and provided a transport property database of various molecules. Monsky et al. [51] illustrated that transvascular transport of macromolecules could be enhanced using vascular endothelial growth factor. Netti et al. [52,53] investigated transvascular transport enhancement by modulating tumor microvascular pressure using periodic or continuous injection of angiotensin II. Recently, a strategy to normalize the tumor vasculature to achieve improved drug delivery throughout the tumor tissue has been proposed [54,55]. This approach aims to remodel the tumor vasculature to functional vasculature of the healthy tissues using antiangiogenic factors which bind to VEGF receptors of tumor-associated endothelial cells.

After extravasation, the drug is transported through the tumor interstitial space against elevated tumor IFP and abnormal ECM structure [33,36,56]. The IFP of a solid tumor stays at an elevated level and sharply decreases at the periphery of the tumor. Due to its importance during drug delivery, physiological changes by elevated tumor IFP have been studied by many researchers [35,53,56]. The IFP of various tumor types varies from 4 to 50 mmHg with an approximate average of 20 mmHg, which is much higher than the IFP of normal tissues, approximately 2 mmHg [35,57]. Drastically higher IFPs of 75~130 mmHg are reported for PDACs [18]. This elevated IFP is thought to result from anomalous characteristics of the tumor vascular structure including high vascular permeability and the lack of a well-developed lymphatic system. The elevated IFP adversely affects the transport of therapeutic agents at several different levels – i) less extravasation of the agents and ii) radially outward interstitial

fluid movement at the periphery of the tumor [8]. Consequently, the elevated IFP contributes to insufficient delivery of drugs to the interior of tumors. Moreover, higher collagen content and consequent dense organization of collagen fibrils result in lower diffusivity of drugs. Thus, transport of drugs is significantly limited in the tumor interstitial space [36,58–60]. A wide variety of methods have been proposed and investigated to enhance the interstitial transport, but the main underlying strategies are either lowering the tumor IFP [61–63] or modulating the tumor ECM structure [36,64]. However, due to the complex interaction involving various physiological parameters, the control or manipulation of the tumor IFP and ECM structure still warrants further research.

Once the drugs are transported through the tumor interstitial space, these should act on tumor cells, but their efficacy may also be limited due to complete or partial drug resistance [65–68]. Multidrug resistance (MDR) is thought to be caused by a group of membrane proteins that extrude cytotoxic molecules, thus maintaining the intracellular drug concentration below effective levels. These proteins belong to the ATP binding cassette (ABC) superfamily of membrane transporters [69], most of which use the energy of ATP hydrolysis for the efflux of drugs (i.e. active transport). This family includes the well-characterized P-glycoprotein (Pgp) encoded by the MDR-1 gene [70,71–75], the multidrug resistance protein (MRP) [76–80] and the mitoxantrone resistance protein (MXR), also known as the breast cancer resistance protein (BCRP) [81–83]. Numerous clinical data imply that MDR phenotypes in tumors are associated with the overexpression of these transporters. Since these transporters have wide recognition patterns of substrates, the overexpression of these proteins will result in multidrug resistance. In addition to the over-expression of these transporter proteins, cellular drug resistance also appears to be mediated by the binding of tumor cells to the ECM [84,85].

The most extensively studied strategy for efficient drug delivery and efficacy is to inhibit drug efflux by modulating the activities of the MDR-associated proteins. This can be achieved by the co-application of MDR modulators with anti-cancer drugs. A wide variety of compounds have been identified as MDR modulators. For example, verapamil, cyclosporine and their derivatives have been investigated in preclinical studies and in some cases have resulted in increased intracellular drug concentration [86–88]. Besides these chemosensitizers, monoclonal antibodies have been studied as potential MDR modulators [89,90]. In addition to the MDR-associated proteins, the membrane lipid has also been investigated as a target for manipulation, as reviewed elsewhere [91]. The alteration of membrane biophysical properties, including membrane fluidity and permeability, could increase or decrease the cellular uptake of drugs [92–94]. Polymeric excipients [95] and transcriptional regulators [96] have also been studied. Although heat shock has been reported to induce MDR in some cancer cells [97,98], an increase in cellular drug uptake and cytotoxicity by ultrasound-induced hyperthermia was reported [99,100]. Unfortunately, delivery of these modulators to the target tumor is as challenging as the drug delivery obstacles we face.

To achieve effective targeted drug delivery, various strategies have been proposed to exploit these pathophysiological characteristics of the TME. Currently, many drug delivery systems, primarily NP-based systems, are designed based on so-called “passive” and “active” targeting strategies, which rely on increased extravasation and ligand–receptor interactions, respectively [101]. The passive targeting is based on the fenestration and prolonged circulation by PEGylation. This is often called the EPR effects since it is caused by the increased vascular permeability of the tumor vasculature [27,28]. However, it has not been shown whether such an EPR effect exists in human tumors. The term “active targeting” is used to describe a strategy to attach ligands on the surface of NPs so that the NPs selectively bind to the target tumor cells or endothelium. Clearly, active targeting becomes effective only after the NPs reach the vicinity of the target tumors. These strategies can result in improved accumulation of NPs at the tumor, but the *in vivo* efficacy of NPs and NP-mediated drug delivery is still significantly impaired [1,10,42]. Only about 5% of the administered dose ends up at the target tumors. Although this may be a 5-fold increase compared to drug molecules, the remaining significant portion of the NPs is still taken up by the RES of the spleen, liver, and lungs as illustrated in Fig. 4B.

Tumor models to simulate TME

Due to the multifaceted complexities of the TME ranging from molecular, genetic, and biological, to chemical and physical parameters, it is very difficult to interpret the efficacy and resistance of drugs and drug delivery systems. All TME parameters are highly dynamic, interconnected and vary spatiotemporally, and may adversely affect the extravasation and interstitial transport of drugs and the subsequent action [13,29]. In order to achieve effective treatment, both drug and delivery system should be designed to properly transport through, and act on, target cells in this complex environment. One critical bottleneck to developing effective targeted delivery systems is a limited quantitative understanding of the *in vivo* transport and action of drugs due to a lack of versatile models capable of rapid systematic study [7,102].

Most widely used tumor models are two dimensional (2D) cell monolayers, often consisting of human cancer cell lines on a substrate. Although these cell lines are valuable tools, their 2D culture environment does not mimic the TME. Thus, the outcome from traditional 2D cell cultures often fails to be indicative of *in vivo* or clinical outcomes. A growing number of studies reported that the physical, chemical and mechanical microenvironments of cancer cells significantly affect cellular behaviors [103–109]. These include changes in cell morphology, gene expression and drug responses. Moreover, interactions of cancer cells with other cell types, ECM molecules, and the interstitial fluid in TME should be properly represented. In order to address these deficiencies, three-dimensional (3D) models including spheroids and engineered tissue scaffolds have been developed [110,111]. The 3D microenvironment and architectural structure provided by these models induce cell morphology, signaling, and gene expression similar to *in vivo* TME.

The simplest *in vitro* 3D tumor model is multicellular tumor spheroid, whereas tumor cells spontaneously aggregate and form a spheroid structure [111–113]. Spheroids can be prepared by several methods including hanging drop method, liquid-overlay cultures, and dynamic bioreactors. In the hanging drop method, cells form spheroids within small droplets of cell suspensions (approximately 20–50 μL) [114]. Due to the surface tension, liquid can maintain droplets when the lid is inverted and cells settled at the bottom of the droplet, air–liquid interface. Liquid-overlay culture prevents cell adhesion to the culture substrates to induce cells to adhere to each other and form aggregates. To prevent adhesion to the culture vessel, the surface can be coated with agarose, poly-2-hydroxyethyl methacrylate (poly-HEMA), or Matrigel inducing cellular aggregations [113,115]. Spheroids can also be formed by culturing in bioreactors, which can provide dynamic conditions preventing cell–substrate contact and promoting cells to form aggregates by either stirring or rotating bioreactors [116]. Nonetheless, these are convenient and relatively simple techniques to simulate *in vivo* solid tumor. Cells within spheroids display cell–cell interactions and produce *in vivo* tumor-like biochemical responses compared to 2D cell culture. However, the lack of interstitial fluid dynamics, which is highly relevant to drug transport within the TME, is one of the major limitations [117,118].

A large number of studies have historically relied on mouse xenograft and allograft (heterotopic and orthotopic) tumor models to test the efficacy of anti-tumor cytotoxic agents against established cancer cell lines. Although these studies have advanced

the field and our knowledge of tumor biology, they are not ideal approaches because the tumors and TME that develop have limited similarity to the human disease. A better approach is to utilize GEMMs that initiate cancer in the correct cell type and at the correct time, to generate a tumor that more closely recapitulates the human TME. However, it is still challenging to obtain mechanistic information regarding drug and NP transport and action in GEMMs. More detailed reviews on animal models used in pre-clinical drug testing can be found elsewhere [119]. The TME of the animal models has many key features that are lacking in 2D and 3D *in vitro* models. However, even animal models often fail to simulate human *in vivo* environments and to provide a mechanistic explanation of the *in vivo* behavior of NPs [102]. This is because of (i) the unknown scaling factors necessary to extrapolate from animal models to human subjects [120], (ii) the mismatch between human cancer cells and mouse matrix environments [121,122], (iii) the difficulties to simulate the heterogeneity of tumor microenvironmental parameters [123,124], and (iv) the inability to independently control these parameters in GEMMs. Thus, a new model system is greatly desired, in which the TME parameters can be systematically and independently controlled, but at the same time the dynamic interactions among the fluids, ECM, cells and NPs are maintained.

In order to address the limitation of *in vitro* static cell culture and mimic a more *in vivo*-like environment, various new cancer cell cultures on microfluidic platforms have been developed as reviewed elsewhere [125–127]. Since this review is focused on drug transport at TME, several models relevant to study drug transport and action are presented in Fig. 5. Most of them are based on

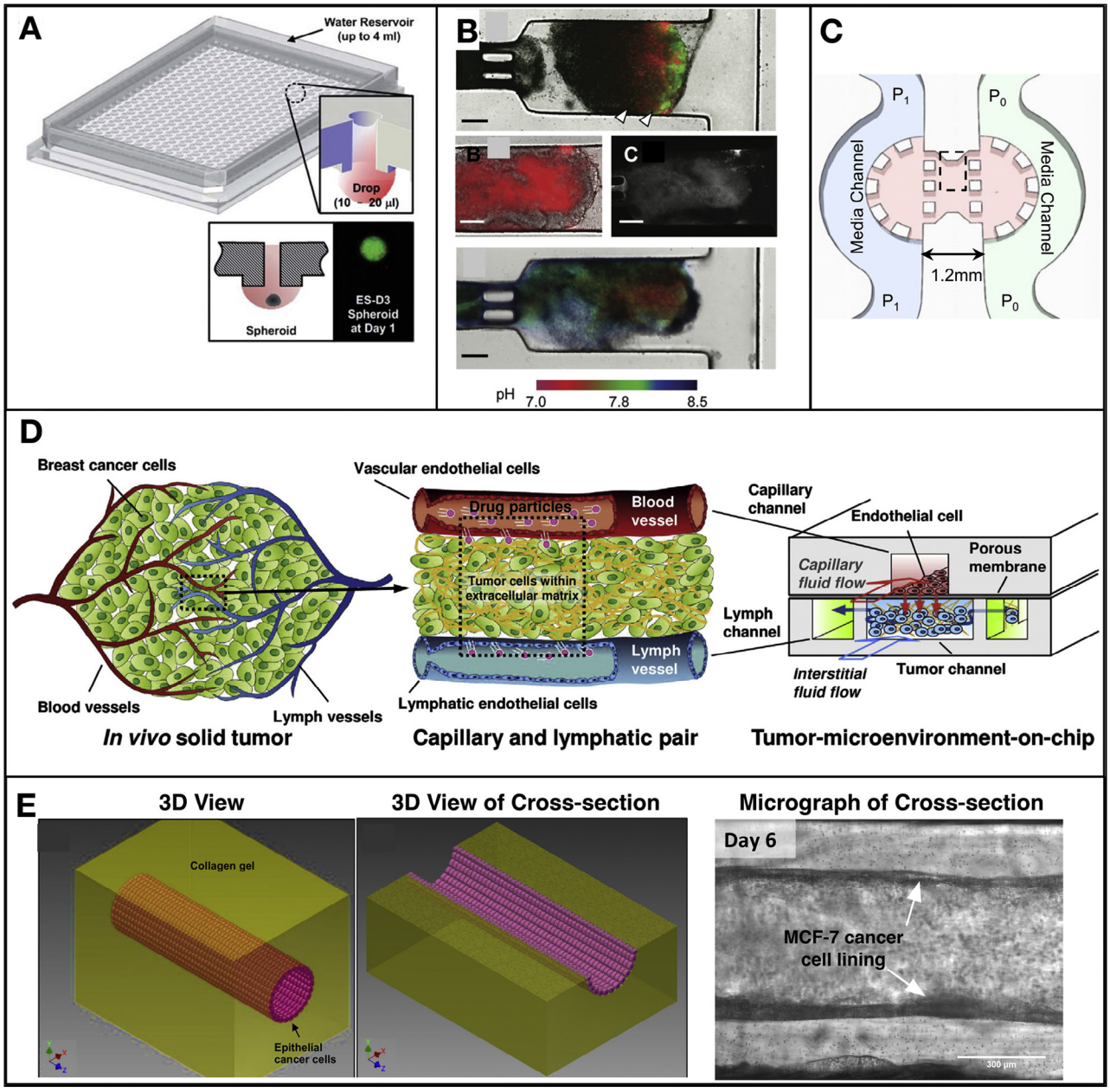

Fig. 5. Recent development of *in vitro* tumor models. (A) Illustration of a high throughput hanging drop spheroid culture array plate and its cross-sectional view. Insets: cartoon of the hanging drop formation and a spheroid [128]. (B) A microfluidic platform to culture tumor cells capable of monitoring cell growth, apoptosis, and pH [129]. (C) A tumor-on-chip platform to study the effects of interstitial fluid pressure gradient [130]. (D) A platform to mimic multiple transport processes at the tumor vasculature and interstitium [131]. (E) A platform to mimic epithelial tumor growth and metastasis from the circular lumen structure [132]. (Figure 5A and 5B were adapted from each reference with permission from The Royal Society of Chemistry.)

microfluidic technology and often called "tumor-on-chip" or "tumor-microenvironment-on-chip." Although not shown, first generation microfluidic cancer cell cultures were 2D cell monolayers cultured on microchannels in the presence of fluid flow exerting shear stress on the cells [133,134]. Specifically, endothelial cells were cultured on a microfluidic channel where shear stress was applied at a controlled manner to study the morphological changes *in vitro* [133]. Epithelial transport characteristics of multiple chemical compounds were also studied under both temporal and spatial chemical gradients [134]. The presence of a cell–fluid interaction was a significant advance from the conventional 2D cell monolayer models, but the lack of 3D environment was the major limitation to be addressed since the cell physiology of 2D models is distinctively different from that of cells cultured in 3D matrices. To address this, 3D culture environments have been created by combining tumor spheroids and tissue engineering technology.

First, tumor spheroid models have been scaled up into a 384-well format hanging drop culture plate for high throughput assay of drug sensitivity as illustrated in Fig. 5A [128]. This model significantly increased the throughput of screening of drug sensitivity for a given spheroid type, and also reported the differences in the drug response when the same types of cells were cultured in either 2D or spheroid format. In order to address the lack of interstitial fluid dynamics, recent studies tried to integrate spheroids into microfluidic platforms where spheroids were subject to interstitial fluid flow [129,135,136]. In these models, cancer cells or spheroids were cultured within polymeric matrices to mimic cell–matrix interactions *in vivo*, allowing for the generation of spatial gradients of growth factors and pH [103,129,137–141]. As illustrated in Fig. 5B, Walsh et al. [129] created the pH gradient of the perfused spheroid culture on a microfluidic platform, and visualized the doxorubicin diffusion through the tumor spheroids.

Although the interstitial fluid flow has been known to hinder drug transport as well as to affect the morphology and migration of cells, it is very difficult to recreate within *in vitro* tumor models, even in microfluidic platforms. Polacheck et al. [130] developed a microfluidic platform to mimic stable pressure gradients and fluid flow across the tumor interstitium as shown in Fig. 5C. In this model, breast cancer cells (MDA-MB-231) were seeded in type I collagen matrix and cultured under the perfusion of interstitial fluid flow created by pressure difference across the matrix. By controlling the pressure of each media channel, the flow rate could be precisely controlled and its effects on cell migration behavior were studied. Cell migration relevant to metastasis and angiogenesis has also been studied using microfluidic platforms [142,143]. In addition, a microfluidic platform has been proposed to mimic hypoxia [144].

All of these 3D tumor models show great promise for mimicking the *in vivo* TME, and ultimately engineered tumors [145,146]. The most significant advantages of these microfluidic 3D models are flexibility and controllability to systematically study the effects of individual TME parameters. However, microfluidics models still warrant further research to create directional cell–matrix and tissue–tissue interactions [147]. Since cells are typically seeded within polymeric scaffolds in these 3D models, their cell–matrix interactions are non-directional and this can greatly affect cell polarity differently from *in vivo* circumstances during cancer development [110,148,149]. Moreover, drug transport *in vivo* is greatly affected by the interfacial phenomena at the tissue–tissue interface, including endothelium–blood, endothelium–interstitium, and interstitium–lymphatic endothelium. These interactions need to be present in the model in order to properly simulate the drug transport *in vivo*.

As shown in Fig. 5D, a T-MOC platform has been developed to recapitulate the complex and multiple transport processes in the TME [131]. Rather than mimicking a whole solid tumor, this platform was designed to recapitulate tumor tissue placed between capillary and lymphatic vessels. It had a 3D structure formed by stacking two PDMS layers of microchannels with a porous membrane inserted between the layers. Endothelial cells were cultured on the porous membrane to mimic the endothelium of the capillary. Along the capillary channel, the drug-suspended medium flowed at physiologically relevant velocity and pressure. After extravasation, drugs entered the center channel of the bottom layer, which simulates the tumor interstitium. In this tumor channel, cancer cells were cultured within a 3D collagen matrix, and the interstitial fluid flowed through the matrix and exerted elevated IFP. Then, the drugs were transported through this 3D tissue structure to reach the cancer cells, and remaining drugs might be drained to two side channels mimicking the lymphatics. Using this T-MOC platform, the transport of NPs and the effects of various TME parameters on the transport were systematically studied, including the vascular pore size, IFP, and collagen content and cell packing density of tumor tissues.

Many epithelial tumors including PDAC and ductal carcinoma in situ (DCIS) originate from round epithelial duct where malignant cells acquire invasive properties and disrupt normal epithelial duct geometry. A T-MOC to mimic this directional cell–cell and cell–matrix interactions is shown in Fig. 5E [132]. A lumen structure was generated along the microfluidic channel using a fluid dynamic phenomenon called "viscous fingering" [150]. First, collagen solution was filled along the microchannel and then culture medium droplets were placed at the inlet port to initiate the viscous fingering. As the lumen formed, the microfluidic chip was incubated to polymerize the collagen and fix the structure. Once the lumen structure was produced, cancer cells were seeded along the lining of the lumen by filling with cancer cell suspension. A micrograph of the cultured tumor with epithelial ductal geometry is shown.

Summary and conclusion

In order to achieve targeted drug delivery, the barriers posed by various aspects of the TME must be overcome to improve the delivery and efficacy of drugs. Identifying a molecular target for delivery systems is a good starting point, but it is not enough to guarantee efficient delivery. To design targeted drug delivery systems, multifaceted aspects of TME should be considered including – i) the dense stroma, hypovascularity, and high IFP of the tumor, which pose biophysical barriers to drug transport; ii) hypoxia, CAF-cancer cell interactions, and genetic instability, which hinder the actions of drugs; and iii) substantial immunosuppression, which is present within the TME. These barriers are highly dynamic and interactive, and spatiotemporally vary during development, progression and treatment. Thus, a systematic study to establish quantitative knowledge of the effects of these parameters on the transport and action of drugs is a must. To mechanistically understand this complex environment, new tumor models are needed that provide systematic control of relevant parameters and rapid/high content analysis of multifaceted drug transport and actions.

In this context, recent efforts to develop new *in vitro* tumor models such as T-MOC will provide a robust and convenient platform to rapidly screen various drug formulations, and to develop new targeted delivery strategies. Although these platforms have been developed to recapitulate the complex TME by culturing cancer cells with stroma cells within 3D extracellular matrices under perfusion, it is still not realistic, nor possible, to design a generic system to fully replicate every aspect of the TME. In order to maximize the benefit of these platforms, the model should be developed to test a specific hypothesis or certain aspects of TME while systematically varying biological, physical and chemical characteristics. Thus, rather than creating another step for drug discovery and screening, it can be used to obtain knowledge and insights, which can be

extrapolated to drug design for pre-clinical animal studies and ultimately humans.

Acknowledgements

This work was partially supported by NIH HHSN261201400021C (BH), CA129287 (KP), CA124586 (SFK), CA075059 (MK), CTR Award (BH, KP) from Indiana CTSI funded in part by UL1 TR000006 from NIH, grants from Walther Cancer Foundation (BH, MK), and an Incentive Grant Program from Purdue University (BH).

Conflict of interest

None.

References

[1] Y.H. Bae, K. Park, Targeted drug delivery to tumors: myths, reality and possibility, J. Control. Release 153 (2011) 198–205.
[2] S.D. Perrault, C. Walkey, T. Jennings, H.C. Fischer, W.C.W. Chan, Mediating tumor targeting efficiency of nanoparticles through design, Nano Lett. 9 (2010) 1909–1915.
[3] R. Tong, H.D. Hemmati, R. Langer, D.S. Kohane, Photoswitchable nanoparticles for triggered tissue penetration and drug delivery, J. Am. Chem. Soc. (2012) doi:10.1021/ja211888a.
[4] C. Wong, T. Stylianopoulos, J. Cui, J. Martin, V.P. Chauhan, W. Jiang, et al., Multistage nanoparticle delivery system for deep penetration into tumor tissue, PNAS 108 (2011) 2426–2431.
[5] G. Bao, S. Mitragotri, S. Tong, Multifunctional nanoparticles for drug delivery and molecular imaging, Annu. Rev. Biomed. Eng. 15 (2013) 253–282.
[6] A. Ozcelikkale, S. Ghosh, B. Han, Multifaceted transport characteristics of nanomedicine: needs for characterization in dynamic environment, Mol. Pharm. (2013) doi:10.1021/mp3005947.
[7] B. Han, Complex transport around tumor: need for realistic in vitro tumor transport model, in: Y. Bae, R. Mrsny, K. Park (Eds.), Cancer Targeted Drug Delivery: An Elusive Dream, Springer Science+Business Media, New York, 2013.
[8] I.K. Kwon, S.C. Lee, B. Han, K. Park, Analysis on the current status of targeted drug delivery to tumors, J. Control. Release 164 (2012) 108–114.
[9] M. Kanapathipillai, A. Brock, D.E. Inger, Nanoparticle targeting of anti-cancer drugs that alter intracellular signaling or influence the tumor microenvironment, Adv. Drug Deliv. Rev. 79 (2014) 107–118.
[10] Y.H. Bae, H.J. Chung, T.G. Park, Nanomaterials for cancer therapy and imaging, Mol. Cells 31 (2011) 295–302.
[11] M. Erkan, S. Hausmann, C.W. Michalski, A.A. Fingerle, M. Dobritz, J. Kleeff, et al., The role of stroma in pancreatic cancer: diagnostic and therapeutic implication, Nat. Rev. Gastroenterol. Hepatol. 9 (2012) 454–467.
[12] D. Mahadevan, D.D.V. Hoff, Tumor-stroma interactions in pancreatic ductal adenocarcinoma, Mol. Cancer Ther. 6 (2007) 1186–1197.
[13] M. Egeblad, M.G. Rasch, V.M. Weaver, Dynamic interplay between the collagen scaffold and tumor evolution, Curr. Opin. Cell Biol. 22 (2010) 697–706.
[14] J. Gore, M. Korc, Pancreatic cancer stroma: friend or foe?, Cancer Cell 25 (2014) 711–712.
[15] D.F. Quail, J.A. Joyce, Microenvironmental regulation of tumor progression and metastasis, Nat. Med. 19 (2013) 1423–1437.
[16] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 64 (2014) 9–29.
[17] S.E. Kern, Molecular genetic alterations in ductal pancreatic adenocarcinomas, Med. Clin. North Am. 84 (2000) 691–695.
[18] P.P. Provenzano, C. Cuevas, A.E. Chang, V.K. Goel, D.D.V. Hoff, S.R. Hingorani, Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma, Cancer Cell 21 (2012) 418–429.
[19] I.M. Stromnes, K.E. DelGiorno, P.D. Greenberg, S.R. Hingorani, Stromal reengineering to treat pancreas cancer, Carcinogenesis 35 (2014) 1451–1460.
[20] S. Jones, X. Zhang, D.W. Parsons, J.C.-H. Lin, R.J. Leary, P. Angenendt, et al., Core signaling pathways in human pancreatic cancers revealed by global genomic analyses, Science 321 (2008) 1801–1806.
[21] P.J. Campbell, S. Yachida, L.J. Mudie, P.J. Stephens, E.D. Pleasance, L.A. Stebbings, et al., The patterns and dynamics of genomic instability in metastatic pancreatic cancer, Nature 467 (2010) 1109–1113.
[22] S. Yachida, S. Jones, I. Bozic, T. Antal, R. Leary, B. Fu, et al., Distant metastasis occurs late during the genetic evolution of pancreatic cancer, Nature 467 (2010) 1114–1117.
[23] J.P. Morris, S.C. Wang, M. Hebrok, K.R.A.S. Hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma, Nat. Rev. Cancer 10 (2010) 683–695.
[24] G. Feldmann, S. Dhara, V. Fendrich, D. Bedja, R. Beaty, M. Mullendore, et al., Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers, Cancer Res. 67 (2007) 2187–2196.
[25] L. Zhu, G. Shi, C.M. Schmidt, R.H. Hruban, S.F. Konieczny, Acinar cells contribute to the molecular heterogeneity of pancreatic intraepithelial neoplasia, Am. J. Pathol. 171 (2007) 263–273.
[26] G. Shi, L. Zhu, Y. Sun, R. Bettencourt, B. Damsz, R.H. Hruban, et al., Loss of the acinar-restricted transcription factor Mist1 accelerates Kras-induced pancreatic intraepithelial neoplasia, Gastroenterology 136 (2009) 1368–1378.
[27] Y. Matsumura, H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS, Cancer Res. 46 (1986) 6387–6392.
[28] J. Fang, H. Nakamura, H. Maeda, The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect, Adv. Drug Deliv. Rev. 63 (2011) 136–151.
[29] A.S. Narang, S. Varia, Role of tumor vascular architecture in drug delivery, Adv. Drug Deliv. Rev. 63 (2011) 640–658.
[30] P.P. Provenzano, S.R. Hingorani, Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer, Br. J. Cancer 108 (2013) 1–8.
[31] J. Gore, K.E. Craven, J.L. Wilson, G.A. Gote, M. Cheng, H.V. Nguyen, et al., TCGA data and patient-derived orthotopic xenografts highlight pancreatic cancer-associated angiogenesis, Oncotarget 6 (10) (2015) 7504–7521.
[32] B.A. Teicher, Tumor models for efficacy determination, Mol. Cancer Ther. 5 (2006) 2435–2443.
[33] E. Brown, T.D. McKee, E. diTomaso, A. Pluen, B. Seed, Y. Boucher, et al., Dynamic imaging of collagen and its modulation in tumors in vivo using second-harmonic generation, Nat. Med. 9 (2003) 796–800.
[34] R. Grantab, S. Sivananthan, I.F. Tannock, The penetration of anticancer drugs through tumor tissue as a function of cellular adhesion and packing density of tumor cells, Cancer Res. 66 (2006) 1033–1039.
[35] C.-H. Heldin, K. Rubin, K. Pietras, A. Östman, High interstitial fluid pressure – an obstacle in cancer therapy, Nat. Rev. Cancer 4 (2004) 806–813.
[36] P.A. Netti, D.A. Berk, M.A. Swartz, A.J. Grodzinsky, R.K. Jain, Role of extracellular matrix assembly in interstitial transport in solid tumors, Cancer Res. 60 (2000) 2497–2503.
[37] M. Korc, Pancreatic cancer-associated stroma production, Am. J. Surg. 194 (2007) S84–S86.
[38] M. Preis, M. Korc, Signaling pathways in pancreatic cancer, Crit. Rev. Eukaryot. Gene Expr. 21 (2011) 115–129.
[39] M.H. Sherman, R.T. Yu, D.D. Engle, N. Ding, A.R. Atkins, H. Tiriac, et al., Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy, Cell 159 (2014) 80–93.
[40] B.C. Ozdemir, T. Pentcheva-Hoang, J.L. Carstens, X. Zheng, C.C. Wu, T.R. Simpson, et al., Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival, Cancer Cell 25 (2014) 719–734.
[41] K. Park, Facing the truth about nanotechnology in drug delivery, ACS Nano 7 (2013) 7442–7447.
[42] N. Bertrand, J.-C. Leroux, The journey of a drug-carrier in the body: an anatomo-physiological perspective, J. Control. Release 161 (2012) 152–163.
[43] M.A. Dobrovolskaia, P. Aggarwal, J.B. Hall, S.E. McNeil, Preclinical studies to understand nanoparticle interaction with immune system and its potential effects on nanoparticle biodistribution, Mol. Pharm. 5 (2008) 487–495.
[44] R. Bansal, E. Post, J.H. Proost, A. de Jager-Krikken, K. Poelstra, J. Parkash, PEGylation improves pharmacokinetic profile, liver uptake and efficacy of interferon gamma in liver fibrosis, J. Control. Release 154 (2011) 233–240.
[45] C.D. Walkey, J.B. Olsen, F. Song, L. Rong, H. Guo, D.W.H. Olsen, et al., Protein corona fingerprinting predicts the cellular interaction of gold and silver nanoparticles, ACS Nano 8 (2014) 2439–2455.
[46] D. Feng, J.A. Nagy, K. Pyne, I. Hammel, H.F. Dvorak, A.M. Dvorak, Pathways of macromolecular extravasation across microvascular endothelium in response to VPF VEGF and other vasoactive mediators, Microcirculation 6 (1999) 23–44.
[47] S.K. Hobbs, W.L. Monsky, F. Yuan, W.G. Roberts, L. Griffith, V.P. Torchilin, et al., Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 4607–4612.
[48] F. Yuan, Transvascular drug delivery in solid tumors, Semin. Radiat. Oncol. 8 (1998) 164–175.
[49] F. Yuan, M. Dellian, D. Fukumura, M. Leunig, D.A. Berk, V.P. Torchilin, et al., Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size, Cancer Res. 55 (1995) 3752–3756.
[50] W.L. Monsky, Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: mammary fat pad versus cranial tumors, Clin. Cancer Res. 8 (2002) 1008–1013.
[51] W. Monsky, F. Yuan, D. Fukumura, V. Torchilin, R.K. Jain, Topical superfusion of vascular endothelial growth factor increases tumor vessel endothelial pore size, Proc. Annu. Meet. Am. Assoc. Cancer Res. 38 (1997) 52.
[52] P.A. Netti, L.M. Hamberg, J.W. Babich, D. Kierstead, W. Graham, G.J. Hunter, et al., Enhancement of fuid filtration across tumor vessels: implication for delivery of macromoloecules, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 3137–3142.
[53] P.A. Netti, L.T. Baxter, Y. Boucher, R. Skalak, R.K. Jain, Time-dependent behavior of interstitial fluid pressure in solid tumors: implications for drug delivery, Cancer Res. 55 (1995) 5451–5458.
[54] V.P. Chauhan, T. Stylianopoulos, J.D. Martin, Z. Popovic, O. Chen, W.S. Kamoun, et al., Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner, Nat. Nanotechnol. 7 (6) (2012) 383–388, doi:10.1038/nnano2012.45.

[55] R.K. Jain, Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy, Science 307 (2005) 58–62.
[56] R.K. Jain, Transport of molecules in the tumor interstitium: a review, Cancer Res. 47 (1987) 3039–3051.
[57] J.R. Less, M.C. Posner, Y. Boucher, D. Borochovitz, N. Wolmark, R.K. Jain, Interstitial hypertension in human breast and colorectoral tumors, Cancer Res. 52 (1992) 6371–6374.
[58] G. Alexandrakis, E. Brown, R.T. Tong, T.D. McKee, R.B. Campbell, Y. Boucher, et al., Two-photon fluorescence correlation microscopy reveals the two-phase nature of transport in tumors, Nat. Med. 10 (2004) 203–207.
[59] A. Pluen, Y. Boucher, S. Ramanujan, T.D. McKee, T. Gohongi, E. diTomaso, et al., Role of tumor-host interactions in interstitial diffusion of macromolecules: cranial vs. subcutaneous tumors, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 4628–4633.
[60] S. Ramanujan, A. Pluen, T.D. McKee, E.B. Brown, Y. Boucher, R.K. Jain, Diffusion and convection in collagen gels: implications for transport in the tumor interstitium, Biophys. J. 83 (2002) 1650–1660.
[61] G. Griffon-Etienne, Y. Boucher, C. Brekken, H.D. Suit, R.K. Jain, Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical implications, Cancer Res. 59 (1999) 3776–3782.
[62] K. Rubin, M. Sjoquist, A.-M. Gustafsson, B. Isaksson, G. Salvessen, R.K. Reed, Lowering of tumoral interstitial fluid pressure by prostaglandin E1 is paralleled by an increased uptake of Cr-EDTA, Int. J. Cancer 86 (2000) 636–643.
[63] A.V. Salnikov, V.V. Iversen, M. Koisti, C. Sundberg, L. Johansson, L.B. Stuhr, et al., Lowering of tumor interstitial fluid pressure specifically augments efficacy of chemotherapy, FASEB J. 17 (2003) 1756–1758.
[64] L. Davies Cde, D.A. Berk, A. Pluen, R.K. Jain, Comparison of IgG diffusion and extracellular matrix composition in rhabdomyosarcomas grown in mice versus in vitro as spheroids reveals the role of host stromal cells, Br. J. Cancer 86 (2002) 1639–1644.
[65] R. Clarke, F. Leonessa, B. Trock, Multidrug resistance/P-glycoprotein and breast cancer: review and meta-analysis, Semin. Oncol. 32 (2005) S9–S15.
[66] W.N. Hait, J.M. Yang, Clinical management of recurrent breast cancer: development of multidrug resistance (MDR) and strategies to circumvent it, Semin. Oncol. 32 (2005) S16–S21.
[67] U. Stein, W. Walther, M. Lemm, H. Naundorf, I. Fichtner, Development and characterization of novel human multidrug resistant mammary carcinoma lines in vitro and in vivo, Int. J. Cancer 72 (1997) 885–891.
[68] K. Wosikowski, J.T. Regis, R. Robey, M. Alvarez, J.T.M. Buters, J.M. Gudas, et al., Normal p53 status and function despite the development of drug resistance in human breast cancer cells, Cell Growth Differ. 6 (1995) 1395–1403.
[69] R. Allikments, L.M. Schrim, A. Hutchinson, V. Romano-Spica, M. Dean, Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database, Hum. Mol. Genet. 5 (1966) 1649–1655.
[70] P.J. Daschner, H.P. Ciolino, C.A. Plouzek, G.C. Yeh, Increased AP-1 activity in drug resistant human breast cancer MCF-7 cells, Breast Cancer Res. Treat. 53 (1999) 229–240.
[71] V.F. Chekhun, G.I. Kulik, O.V. Yurchenko, V.P. Tryndyak, I.N. Todor, L.S. Luniv, et al., Role of DNA hypomethylation in the development of the resistance to doxorubicin in human MCF-7 breast adenocarcinoma cells, Cancer Lett. 231 (2006) 87–93.
[72] M.M. Gottesman, I. Pastan, Biochemistry of multidrug resistance mediated by the multidrug transporter, Annu. Rev. Biochem. 62 (1993) 385–427.
[73] J. Jin, F.-P. Wang, H. Wei, G. Liu, Reversal of multidrug resistance of cancer through inhibition of P-glycoprotein by 5-bromoterandrine, Cancer Chemother. Pharmacol. 55 (2005) 179–188.
[74] E. Mechetner, A. Kyshtoobayeva, S. Zonis, H. Kim, R. Stroup, R. Garcia, et al., Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with in vitro resistance to taxol and doxorubicin, Clin. Cancer Res. 4 (1998) 389–398.
[75] M.R. Vredenburg, I. Ojima, J. Veith, P. Pera, K. Kee, F. Cabral, et al., Effects of orally active taxanes on P-glycoprotein modulation and colon and breast carcinoma drug resistance, J. Natl. Cancer Inst. 93 (2001) 1234–1245.
[76] A. Aszalos, K. Thompson, J.J. Yin, D.D. Ross, Combination of P-glycoprotein blockers, verapamil, PSC833 and cremophor act differently on the multidrug resistance associated protein (MRP) and on P-glycoprogein (Pgp), Anticancer Res. 19 (1999) 1053–1064.
[77] M.C. de Jong, J.W. Slootstra, G.L. Scheffer, A.B. Schroeijers, W.C. Puijk, R. Dinkelberg, et al., Peptide transport by the multidrug resistance protein MRP1, Cancer Res. 61 (2001) 2552–2557.
[78] M.J. Ferreira, N. Gyemant, A.M. Madureira, M. Tanaka, K. Koos, R. Didziapetris, et al., The effects of jatrophane derivatives on the reversion of MDR1- and MRP-mediated multidrug resistance in the MDA-MB-231 (HTB-26) cell line, Anticancer Res. 25 (2005) 4173–4178.
[79] M. Filipits, R.W. Suchomel, G. Dekan, K. Haider, G. Valdimarsson, D. Depisch, et al., MRP and MDR1 gene expression in primary breast carcinomas, Clin. Cancer Res. 2 (1996) 1231–1237.
[80] M. Muller, C. Meijer, G.J.R. Zaman, P. Borst, R.J. Scheper, N.H. Mulder, et al., Overexpression of the gene encoding the multidrug resistance-associated protein results in increased ATP-dependent glutathione S-conjugate transport, PNAS 91 (1994) 13033–13037.
[81] M. de Bruin, K. Miyake, T. Litman, R. Robey, S.E. Bates, Reversal of resistance by GF120918 in cell lines expressing the ABC half-transporter, MXR, Cancer Lett. 146 (1999) 117–126.
[82] T. Litman, M. Brangi, E. Hudson, P. Fetsch, A. Abati, D.D. Ross, et al., The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2), J. Cell Sci. 113 (2000) 2011–2021.
[83] R. Robey, W.Y. Medina-Perez, K. Nishiyama, T. Lahusen, K. Miyake, T. Litman, et al., Overexpression of the ATP-binding cassette half-transporter, ABCG2 (MXR/BCRP/ABCP1), in flavopiridol-resistant human breast cancer cells, Clin. Cancer Res. 7 (2001) 145–152.
[84] W.S. Dalton, The tumor microenvironment: focus on myeloma, Cancer Treat. Rev. 29 (2003) 11–19.
[85] C. Jean, P. Gravelle, J.-J. Fournie, G. Laurent, Influence of stress on extracellular matrix and integrin biology, Oncogene 30 (2011) 2697–2706.
[86] S.K. Chambers, W.N. Hait, B.M. Kacinski, Enhancement of anthracycline growth inhibition in parent and multidrug-resistant Chinese hamster ovary cells by cyclosporin A and its analogues, Cancer Res. 49 (1989) 6275–6279.
[87] J.M. Ford, J.M. Yang, W.N. Hait, Effect of buthionine sulfoximine on toxicitiy of verapamil and doxorubicin to multidrug resistant cells and to mice, Cancer Res. 51 (1991) 67–72.
[88] J.M. Yang, S. Goldenberg, M.M. Gottesman, Characteristics of P338/VMDRC.04, a simple, sensitive model for studying P-glycoprotein antagonists, Cancer Res. 54 (1994) 730–737.
[89] Y. Mano, H. Suzuki, T. Terasaki, Kinetic analysis of the disposition of MRK16, an anti-P-glycoprotein monoclonal antibody, in tumors: comparison between in vitro and in vivo disposition, J. Pharmacol. Exp. Ther. 283 (1997) 391–401.
[90] M. Naito, H. Tsuge, C. Kuroko, Enhancement of cellular accumulation of cyclosporine by anti-P-glycoprotein monoclonal antibody MRK-16 and synergistic modulation of multidrug resistance, J. Natl. Cancer Inst. 85 (1993) 311–316.
[91] A.B. Hendrich, K. Michalak, Lipids as a target for drugs modulating multidrug resistance of cancer cells, Curr. Drug Targets 4 (2003) 23–30.
[92] G. Breuzard, O. Piot, J.F. Angiboust, M. Manfait, L. Candeil, M. Del Rio, et al., Changes in adsorption and permeability of mitoxantrone on plasma membrane of BCRP/MXR resistant cells, Biochem. Biophys. Res. Commun. 329 (2005) 64–70.
[93] R. Callaghan, A. Stafford, R.M. Epand, Increased accumulation of drugs in a multidrug resistance cell line by alteration of membrane biophysical properties, Biochim. Biophys. Acta 1175 (1993) 277–282.
[94] F.A. Sinicrope, P.K. Dudeja, B.M. Bissonnette, A.R. Safa, T.A. Brasitus, Modulation of P-glycoprotein-mediated drug transport by alterations in lipid fluidity of rat liver canalicular membrane vesicles, J. Biol. Chem. 267 (1992) 24995–25002.
[95] M. Tijerina, K.D. Fowers, P. Kopeckova, J. Kopecek, Chronic exposure of human ovarian carcinoma cells to free or HPMA copolymer-bound mesochlorin e6 does not induce P-glycoprotein-mediated multidrug resistance, Biomaterials 21 (2000) 2203–2210.
[96] P. Orth, D. Schnappinger, W. Hillen, W. Saenger, W. Hinriches, Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system, Nat. Struct. Biol. 7 (2000) 215–219.
[97] J.-Z. Cheng, R. Sharma, Y. Yang, S.S. Singhal, A. Sharma, M.K. Saini, et al., Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress, J. Biol. Chem. 276 (2001) 41213–41223.
[98] D. Szabo, H. Keyzer, H.E. Kaiser, J. Molnar, Reversal of multidrug resistance of tumor cells, Anticancer Res. 20 (2000) 4261–4274.
[99] Y. Liu, C.-W. Cho, X. Yan, T.K. Henthorn, K.O. Lillehei, W.N. Cobb, et al., Ultrasound-induced hyperthermia increases cellular uptake and cytotoxicity of P-glycoprotein substrates in multi-drug resistant cells, Pharm. Res. 18 (2001) 1225–1261.
[100] Y. Liu, K.O. Lillehei, W.N. Cobb, U. Christians, K. Ng, Overcoming MDR by ultrasound-induced hyperthermia and P-glycoprotein modulation, Biochem. Biophys. Res. Commun. 289 (2001) 62–68.
[101] F. Danhier, O. Feron, V. Preat, To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery, J. Control. Release 148 (2010) 135–146.
[102] G. Damia, M. D'Incalci, Contemporary pre-clinical development of anticancer agents – what are the optimal preclinical models?, Eur. J. Cancer 45 (2009) 2768–2781.
[103] D. Loessner, K.S. Stok, M.P. Lutolf, D.W. Hutmacher, J.A. Clements, S.C. Rizzi, Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells, Biomaterials 31 (2010) 8494–8506.
[104] F. Pampaloni, E.G. Reynaud, E.H.K. Stelzer, The third dimension bridges the gap between cell culture and live tissue, Nat. Rev. Mol. Cell Biol. 8 (2007) 839–845.
[105] M.V. Fournier, K.J. Martin, P.A. Kenny, K. Xhaja, I. Bosch, P. Yaswen, et al., Gene expression signature in organized and growth-arrested mammary acini predicts good outcome in breast cancer, Cancer Res. 66 (2006) 7095–7102.
[106] P.A. Kenny, G.Y. Lee, C.A. Myers, R.M. Neve, J.R. Semeiks, P.T. Spellman, et al., The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression, Mol. Oncol. 1 (2007) 84–96.
[107] K. Tanner, H. Mori, R. Mroue, A. Bruni-Cardoso, M.J. Bissell, Coherent angular motion in the establishment of multicellular architecture of glandular tissues, PNAS 109 (2012) 1973–1978.
[108] B. Weigelt, M.J. Bissell, Unraveling the microenvironmental influences on the normal mammary gland and breast cancer, Semin. Cancer Biol. 18 (2008) 311–321.
[109] B. Weigelt, A.T. Lo, C.C. Park, J.W. Gray, M.J. Bissell, HER2 signaling pathway activation and response of breast cancer cells to HER2-targeting agents is

dependent strongly on the 3D microenvironment, Breast Cancer Res. Treat. 122 (2010) 35–43.
[110] K.M. Yamada, E. Cukierman, Modeling tissue morphogenesis and cancer in 3D, Cell 130 (2007) 601–610.
[111] J.B. Kim, Three-dimensional tissue culture models in cancer biology, Semin. Cancer Biol. 15 (2005) 365–377.
[112] G. Hamilton, Multicellular spheroids as an in vitro tumor model, Cancer Lett. 131 (1998) 29–34.
[113] J. Friedrich, C. Seidel, R. Ebner, L.A. Kunz-Schughart, Spheroid-based drug screen: considerations and practical approach, Nat. Protoc. 4 (2009) 309–324.
[114] J.M. Kelm, N.E. Timmins, C.J. Brown, M. Fussenegger, L.K. Nielsen, Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types, Biotechnol. Bioeng. 83 (2003) 173–180.
[115] Y.T. Phung, D. Barbone, V.C. Broaddus, M. Ho, Rapid generation of in vitro multicellular spheroids for the study of monoclonal antibody therapy, J. Cancer 2 (2011) 507–514.
[116] K. Nakamura, H. Kuga, T. Morisaki, E. Baba, N. Sato, K. Mizumoto, et al., Simulated microgravity culture system for a 3-D carcinoma tissue model, Biotechniques 33 (2002) 1074–1076.
[117] C.P. Ng, M.A. Swartz, Fibroblast alignment under interstitial fluid flow using a novel 3-D tissue culture model, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H1771–H1777.
[118] M.A. Swartz, A.W. Lund, Lymphatic and interstitial flow in the tumour microenviornment: linking mechanobiology with immunity, Nat. Rev. Cancer 12 (2012) 210–219.
[119] H. HogenEsch, A.Y. Nikitin, Challenges in pre-clinical testing of anti-cancer drugs in cell culture and in animal models, J. Control. Release 164 (2) (2012) 183–186, doi:10.1016/j.jconrel.2012.02.031.
[120] A.T. Florence, Targeting nanoparticles: the constraints of physical laws and physical barriers, J. Control. Release 164 (2) (2012) 115–124.
[121] O.J. Becher, E.C. Holland, Genetically engineered models have advantages over xenografts for preclinical studies, Cancer Res. 66 (2006) 3355–3358.
[122] K.K. Frese, D.A. Tuveson, Maximizing mouse cancer models, Nat. Rev. Cancer 7 (2007) 645–658.
[123] A. Marusyk, V. Almendro, K. Polyak, Intra-tumour heterogeneity: a looking glass for cancer, Nat. Rev. Cancer 12 (2012) 323–334.
[124] N. Samuel, T.J. Hudson, The molecular and cellular heterogeneity of pancreatic ductal adenocarcinoma, Nat. Rev. Gastroenterol. Hepatol. 9 (2012) 77–87.
[125] E.K. Sackmann, A.L. Fulton, D.J. Beebe, The present and future role of microfluidics in biomedical research, Nature 507 (2014) 181–189.
[126] D. Choudhury, X. Mo, C. Iliescu, L.L. Tan, W.H. Tong, H. Yu, Exploitation of physical and chemical constraints for three-dimensional microtissue construction in microfluidics, Biomicrofluidics 5 (2011) 022203.
[127] P.M. Valencia, O.C. Farokhzad, R. Karnik, R. Langer, Microfluidic technologies for accelerating the clinical translation of nanoparticles, Nat. Nanotechnol. 7 (2012) 623–629.
[128] Y.C. Tung, A.Y. Hsiao, S.G. Allen, Y. Torisawa, M. Ho, S. Takayama, High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array, Analyst 136 (2011) 473–478.
[129] C.L. Walsh, B.M. Babin, R.W. Kasinskas, J.A. Foster, M.J. McGarry, A multipurpose microfluidics device designed to mimic microenviornment gradients and develop targeted cancer therapeutics, Lab Chip 9 (2009) 545–554.
[130] W.J. Polacheck, J.L. Charest, R.D. Kamm, Interstitial flow influences direction of tumor cell migration through competing mechanisms, PNAS 108 (2011) 11115–11120.
[131] B. Kwak, A. Ozcelikkale, C.S. Shin, K. Park, B. Han, Simulation of complex transport of nanoparticles around a tumor using tumor-microenvironment-on-chip, J. Control. Release 194 (2014) 157–167.
[132] V. Noe-Kim, A. Ozcelikkale, B. Han, An in vitro microfluidic tumor model to mimic ductal carninoma in situ, Proceedings of the 2015 Summer Biomechanics, Bioengineering and Biotransport Conference, SB3C2015-673, 2015.
[133] J.W. Song, W. Gu, N. Futai, K.A. Warner, J.E. Nor, S. Takayama, Computer-controlled microcirculatory support system for endothelial cell culture and shearing, Anal. Chem. 77 (2005) 3993–3999.
[134] B.-J. Jin, E.-A. Ko, W. Namkung, A.S. Verkman, Microfluidics platform for single-shot dose-response analysis of chloride channel-modulating compounds, Lab Chip 13 (2014) 3862–3867.
[135] C.S. Shin, B. Kwak, B. Han, K. Park, Development of an in vitro 3D tumor model to study therapeutic efficacy of an anticaner drug, Mol. Pharm. 10 (2013) 2167–2175.
[136] A. Albanese, A.K. Lam, E.A. Sykes, J.V. Rocheleau, W.C.W. Chan, Tumour-on-chip provides an optical window into nanoparticle tissue transport, Nat. Commun. 4 (2013).
[137] U. Haessler, Y. Kalinin, M.A. Swartz, M. Wu, An agarose-based microfluidic platform with a gradient buffer for 3D chemotaxis studies, Biomed. Microdevices 11 (2009) 827–835.
[138] S. Kim, H.J. Kim, N.L. Jeon, Biological applications of microfluidic gradient devices, Integr. Biol. (Camb.) 2 (2010) 584–603.
[139] C. Fischbach, R. Chen, T. Matsumoto, T. Schmelzle, J.S. Brugge, P.J. Polverini, et al., Engineering tumors with 3D scaffolds, Nat. Methods 4 (2007) 855–860.
[140] A.Y. Hsiao, Y.S. Torisawa, Y.C. Tung, S. Sud, R.S. Taichman, K.J. Pienta, et al., Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids, Biomaterials 30 (2009) 3020–3027.
[141] C.P. Huang, J. Lu, H. Seon, A.P. Lee, L.A. Flanagan, H.Y. Kim, et al., Engineering microscale cellular niches for three-dimensional multicellular co-cultures, Lab Chip 9 (2009) 1740–1748.
[142] I.K. Zervantonakis, S.K. Hughes-Alford, J.L. Charest, J.S. Condeelis, F.B. Gertler, R.D. Kamm, Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function, PNAS 109 (2012) 13515–13520.
[143] S. Bersini, J.S. Jeon, G. Dubini, C. Arrigoni, S. Chung, J.L. Charest, et al., A microfluidic 3D in vitro model for specificity of breast cancer metastasis to bone, Biomaterials 35 (2014) 2454–2461.
[144] K. Funamoto, I.K. Zervantonakis, Y. Liu, C.J. Ochs, C. Kim, R.D. Kamm, A novel microfluidic platform for high-resolution imaging of a three-dimensional cell culture under a controlled hypoxic environment, Lab Chip 12 (2012) 4855.
[145] D.W. Hutmacher, E. Cukierman, Engineering of tumor microenvironments, Adv. Drug Deliv. Rev. 79-80 (2014) 1–2.
[146] C.M. Ghajar, M.J. Bissell, Tumor engineering: the other face of tissue engineering, Tissue Eng. Part A 16 (2010) 2153–2156.
[147] K. Shen, S. Luk, D.F. Hicks, J.S. Elman, S. Bohr, Y. Iwamoto, et al., Resolving cancer-stroma interfacial signalling and interventions with micropatterned tumour-stromal assays, Nat. Commun. 5 (2014) 5662.
[148] J.I. Lopez, J.K. Mouw, W.M. Weaver, Biomechanical regulation of cell orientation and fate, Oncogene 27 (2008) 6981–6993.
[149] D.E. Ingber, Can cancer be reversed by engineering the tumor microenvironment?, Semin. Cancer Biol. 18 (2006) 356–364.
[150] L.L. Bischel, E.W.K. Young, B.R. Mader, D.J. Beebe, Tubeless microfluidic angiogenesis assay with three-dimensional endothelial-lined microvessels, Biomaterials 34 (2013) 1471–1477.