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Miniaturized pre-clinical cancer models as research and diagnostic tools ☆

Maria Håkanson [a], Edna Cukierman [b,*], Mirren Charnley [c,**]

[a] CSEM SA, Section for Micro-Diagnostics, 7302 Landquart, Switzerland
[b] Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA 19111, USA
[c] Centre for Micro-Photonics and Industrial Research Institute Swinburne, Swinburne University of Technology, Victoria 3122, Australia

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ABSTRACT

Cancer is one of the most common causes of death worldwide. Consequently, important resources are directed towards bettering treatments and outcomes. Cancer is difficult to treat due to its heterogeneity, plasticity and frequent drug resistance. New treatment strategies should strive for personalized approaches. These should target neoplastic and/or activated microenvironmental heterogeneity and plasticity without triggering resistance and spare host cells. In this review, the putative use of increasingly physiologically relevant microfabricated cell-culturing systems intended for drug development is discussed. There are two main reasons for the use of miniaturized systems. First, scaling down model size allows for high control of microenvironmental cues enabling more predictive outcomes. Second, miniaturization reduces reagent consumption, thus facilitating combinatorial approaches with little effort and enables the application of scarce materials, such as patient-derived samples. This review aims to give an overview of the state-of-the-art of such systems while predicting their application in cancer drug development.

Contents

Abbreviations: μCCA, microscale cell culture analog; CAM-DR, cell-adhesion mediated drug resistance; CTCs, circulating tumor cells; ECM, extracellular matrix; HGF, hepatocyte growth factor; Hh, Hedgehog; MSCs, mesenchymal stem cells; VEGF, vascular endothelial cells growth factor.

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* Corresponding author. Tel.: +1 215 214 4218.
** Corresponding author. Tel.: +61 3 9214 8008.
E-mail addresses: Edna.Cukierman@FCCC.edu (E. Cukierman), mcharnley@swin.edu.au (M. Charnley).

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1. Introduction

Cancer remains a leading cause of death, for example, it is predicted to be responsible for one of every four deaths in the United States for 2013 [1]. Thus, it is not surprising that the majority of drug candidates in development today are aimed at stalling or eliminating cancer [2]. However, cancer drug development is costly, as it suffers from a very low success rate. For new compounds in clinical development, the success rate is three times lower for cancer drugs than for drugs treating cardiovascular diseases [3]. Several factors complicate the development of drugs that can effectively treat cancer. The high heterogeneity and plasticity of an ever-changing dynamic disease [4] make it difficult to develop drugs that are widely applicable and that have low side-effects. Resistance to treatment ensues due to both repetitive treatment [5] and the unique cues derived from the tumor-specific microenvironment. Development of more specific drugs, which are less likely to induce resistance, requires not only smart drug design but also more predictive pre-clinical tests [6,7]. Together with the development of personalized approaches, treatments specifically targeting the interaction between cancer cells and tumor-associated microenvironment may enable us to overcome the hurdle posed by the heterogeneity in cancer [8–10].

There is extensive evidence demonstrating the importance of the tumor microenvironment on cancer progression [11–13] and treatment outcome [14,15]. In fact, the tumor-associated stroma can play a role as a pro-tumorigenic environment [16], a drug desensitization setting [6,17–19] or a drug penetration barrier [20–22], which complicates any therapeutic approach. To this end, the specific cross talk between a given cancer and its stroma will have to be defined for each cancer type (and perhaps for each patient) in order to achieve effective therapeutic targeting [23]. Consequently, in vitro models that better reflect the in vivo environment may provide a more accurate indication of patient outcome [24–27]. The parameters that are critical for a functional model have been studied in-depth (Fig. 1). For example, culture of cells in a 3D environment is crucial for several aspects of cell behavior [28–30] including the regulation of growth in cancerous [31–33] and migratory cells [13,34,35] as well as for cell–cell interaction-dependent processes such as morphogenesis [32,36]. More relevant culture systems not only include adapting the culture environment but also require advances in the types of cells that are used. Established and immortalized cell lines are typically applied due to their ease of use, reproducibility, and availability. However, many of these cell lines are often altered in comparison to the corresponding primary cells or original tumors on both phenotypic and genotypic levels [37]. Therefore, moving to the use of primary cells (although often not very practical) is one way of increasing predictivity of in vitro assays [38,39]. However, due to the high level of heterogeneity in neoplasias resulting in differing drug responses even between patients with the same diagnosis, it may sometimes be necessary to use patient-derived cells to ensure a higher level of in vivo mimicry and hence increase the predictive value of personalized assays [40,41]. As heterotypic cell interactions are very fundamental for the function of certain tissues [42], co-culture methods including multiple cell types per model system are another means of increasing relevance [43–45].

Today there are a vast number of approaches, using microfabrication and novel scaffold materials, to develop new (i.e., 3D) cell culture platforms that recapitulate the characteristics of the in vivo environment [13,44,46–52]. These models have been crucial for the understanding of the role of the in vivo environment on the behavior of normal and malignant cells [53] and are currently making the first steps into drug development [54]. Microfabricated culture systems are advantageous as they offer control of the culture environment with high reproducibility at the level of single cells [55]. Thus a high control of the cell culture

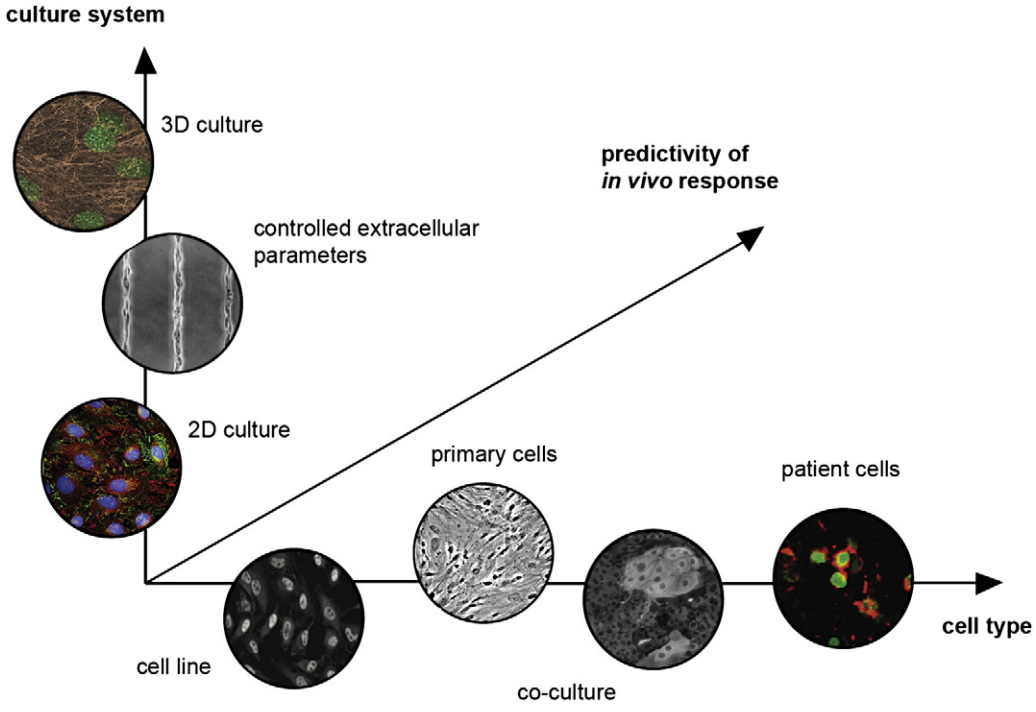

Fig. 1. Stages in the progression towards more relevant models in cell-based assays. The predictive value of drug development should theoretically improve when increasingly (in vivo) relevant models are used. This graph outlines some directions taken in cell culture development to improve these predictive values. Two parameters, cell type and culture system, are plotted in an x–y graph. The linear curve thereby explains the hypothetical increase in predictability of a cell culture system with the alteration of these parameters. Along the x-axis, some examples of cell-types that have a higher relevance than cell lines in monoculture are plotted. This ranges from primary cells [87] to co-culture [243] to patient-derived materials [41], which is envisioned to be the most relevant model system. Systems that enable the culture and analysis of patient cells can be used for tailored treatment, which holds a high promise for the improvement of cancer therapy. On the y-axis, the culture platforms of different relevance are plotted. Depending on the model, a system with controlled extrinsic parameters may increase the relevance compared to standard 2D culture platforms [28,244]. For example, long 2D patterns make it possible to control cell growth of endothelial cells as capillary structures in 2D [26]. Platforms that enable 3D culture and recapitulate the organization of cells in vivo are probably among the most relevant [13,33,57,87].

environment can be obtained by tightly regulating cell shape, dimensionality, adhesive surfaces/ligands, amount of cell–cell contacts and the level and nature of provided soluble factors [47,51,56–58].

Since the early exploration of microfabricated and/or microfluidic systems for cell studies in the 1990s [59], it has been predicted that this research area will contribute to improved systems in drug development [60,61]. Microtechnological approaches have highlighted the importance of the cell organization on a single-cell level [26,58,62], as well as of solute gradients and flow [63–65] for cell behavior and drug response [66]. In spite of a slow translation from the bioengineering labs to the application among biologists and clinical researchers, the motivation to improve the in vitro tools in pre-clinical development is now high, providing a greater impetus for new models to be evaluated. More predictive models could cut the costs in drug development, as more compounds could be ruled in or out before conducting expensive animal and patient studies [67]. Clinical trials alone constitute the largest single cost in the drug development process. For the same reason, high-fidelity cell-based assays have been increasingly used in the last decade [68,69] both in target-validation and pre-clinical screening [70]. The advantage of cell-based over molecular assays is that they better represent the site of action of a drug including more of the in vivo complexity. Thereby, unpredicted targets and evidence of possible negative side effects may be discovered at an early stage.

We now stand at a point where the general improvement offered by organotypic cell culture models is widely accepted. However, these models still need to be more extensively evaluated to understand their power in drug development. This is not a trivial task. For example, we need to understand the model complexity needed for a certain disease area. In some instances, increased predictivity in a model may be achieved by simply switching from 2D culture to 3D culture or by replacing established cell lines with primary cells [54]. In other cases, the parallel adaptation of the microenvironment may be crucial for the relevance of other models [32]. To establish this knowledge base, a joint interdisciplinary effort is required involving basic and clinical researchers, bioengineers, pharmacological developers and automation engineers, among others. For example, a recent systematic evaluation of different culture techniques at Roche was proven to be efficient at determining the optimal functional models for toxicological studies in hepatocytes [25].

This review aims to highlight the current clinically relevant information that could be obtained using miniaturized (i.e., microfabricated/microfluidics systems) cell models. For example, we will discuss the possibility of mimicking the microenvironment to allow, e.g., stromal-dependent and other heterotypic cell contact-dependent drug responses to be discovered. In general, these systems also offer a unique opportunity for an in-depth mechanistic understanding of specific cellular behaviors crucial in cancer, such as cell transformation and metastasis. In addition, miniaturized models also have great potential in the field of personalized treatment as diagnostic tools, given the minimal cell numbers needed per sample. Finally, some models will also allow for combinatorial studies of drugs while altering microenvironmental factors, which should be advantageous in the development of targeted personalized strategies [6].

2. The importance of the tumor microenvironment in disease progression and treatment response

Most eukaryotic cells exist in a complex environment in which they are in contact with surrounding cells, soluble factors and/or extracellular matrix (ECM). These interactions are important for the continuous regulation of homeostatic cell dynamics, which is clearly altered during tumor development and progression. There are currently several lines of evidence demonstrating the crucial role of the microenvironment in cancer progression [71], dissemination and metastasis [13,72]. Hence, even though cancer may originate from genetic mutations, its progression may also be promoted by certain physiological conditions in the cancer microenvironment [73]. In fact, some investigators go as far as to suggest that looking for differences between normal and cancerous cells is unproductive [74], while others suggest treating the activated tumor-microenvironment as a means for cancer erosion [75] or evaluating the levels of stromal activation and using these as predictive factors analogous to classic cancer staging or grading [23,50,76]. Although originally proposed more than a century ago [77,78], the importance of the tumor microenvironment has only recently become well accepted [79–83]. The interaction between cancer cells and their microenvironment reportedly affects the outcome of treatment, [15] and we have only just started to apply this knowledge in diagnosis and treatment [84]. For an improved translation from pre-clinical testing to patient outcome, test models that better reflect the complexity of the cancer microenvironment may become crucial [6,20,24].

The vast majority (about 90%) of human cancers are carcinomas, malignant tumors of epithelial origin. For example, the first transformed (cancerous) epithelial cells at the primary site of breast cancer, a common type of carcinoma, are initially surrounded by healthy epithelial cells and by an epithelial cell-derived and laminin rich ECM known as basement membrane. As the tumor progresses, this “normal” basement membrane, that physically separates the epithelial from the connective (mesenchymal) tissue, is degraded thus facilitating a direct interaction (as opposed to indirectly paracrine signaling) between cancer cells and the tumor-associated mesenchymal stroma [85]. With this, the tumor cells are exposed to a changing microenvironment. Initially, the environment contains epithelial cells and predominantly epithelial ECM-associated proteins, such as collagen IV and laminin. Subsequently, the cancer cells directly interact with a completely different niche, the tumor-associated stroma, which is composed of activated mesenchymal cells (e.g., myofibroblasts), which produce and alter collagen I rich ECM with a peculiar topography [13,80,86,87]. Changes also include the stiffening of the tumor stroma by increased collagen deposition and crosslinking [88]. Hence, tumorigenic process itself is thought to be responsible for the activation of the pro-tumorigenic stroma [80,85]. Reciprocally, it is well accepted that such a tumor-activated mesenchymal microenvironment effectively promotes tumorigenesis in a positive feed-back loop [89,90]. The activated myofibroblast-rich stroma has been shown to support cancer progression including cell proliferation and metastasis [91–97]. Parameters that modulate cancer progression include, but are not limited to, integrin signaling [8], matrix rigidity [88], hypoxia [98] and signaling from stromal cells [99]. Interestingly, there is not a clear-cut division in cause and consequence in these tumor–stroma relationships. Moreover, in vitro experimentation has shown that, on the one hand, normal fibroblasts (and recruited mesenchymal stem cells as well as others) undergo conversion to myofibroblasts in response to tumor cells [100], but these changes can also be induced as a result of a positive stromal feedback stimulated by the tumor-altered mesenchymal ECMs [87].

In addition to its effects on cancer progression, the microenvironment also alters the overall response to cancer drug treatment [6,101], for example, by limiting drug penetration through the tumor-associated stroma [20,102] or by reducing drug effectiveness due to increased cell density and consequently reduced metabolism and proliferation, which lowers the susceptibility of the tumor cells to the drug in question [62,103]. A more complex factor is the biochemical signaling imparted by the tumor microenvironment known as cell-adhesion mediated drug resistance (CAM-DR) [15,104]. This drug treatment cancer protective effect originates in the adhesion of cells to both other cells and ECM components at the tumor site (Fig. 2A). The crucial effects of the microenvironment on drug response have been demonstrated in various systems [105]. These include (among others) using cell-derived ECM matrices in vitro while validating the observations in vivo using animal models and human samples [12,13,87,106]. The interesting observations linking cancer progression with increasing drug resistance were observed in several neoplasias such as ovarian cancer and melanoma. In the first case, a tumor-ECM cooperative effect was found as an over-

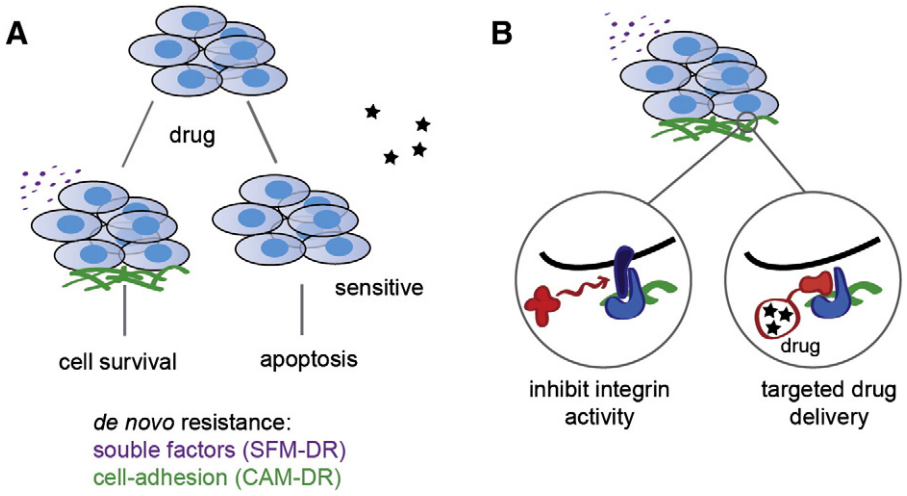

Fig. 2. The role of the microenvironment in drug response and new treatment strategies. Factors present in the tumor microenvironment induce environment-mediated drug resistance (EMDR) by two primary mechanisms: soluble factor-mediated drug resistance (SFM-DR) and cell adhesion-mediated drug resistance (CAM-DR) (A). When the cancer is treated for the first time, most tumor cells respond to the drugs. However, the interaction with microenvironmental factors can give enough protective signaling for some of the cells to survive therapy and eventually to repopulate the tumor with resistant cells. Therefore, therapeutic strategies that disrupt EMDR pathways would reduce the level of surviving cells and prevent the emergence of acquired resistance [6,15]. Cell adhesion via integrins is fundamental for cell survival, behavior and cell migration. Therefore, these receptors are central in the development of new tumor–stromal interaction disrupting drugs (B). Inhibiting specific integrins can reduce the effect of CAM-DR and block additional stromal processes such as angiogenesis [128–131] (left). On the other hand, the specific tumor microenvironment can also be used for targeted delivery of drugs by, for example, recognition of a cancer-specific fibronectin [245] (right).

expression of collagen VI correlated with ovarian cancer grade, while adhesion of tumor cells to this substrate in vitro mediated CAM-DR [107]. In the second case, stromal secretion of hepatocyte growth factor (HGF) triggers activation of its receptor MET in melanoma thus reactivating MAPK and PI3K/Akt, which in turn results in an acquired resistance to RAF inhibition [19]. Interestingly, the signaling mechanism responsible for these types of effects comprises a microenvironmental regulated RAF-inhibitor resistance in BRAFV600E mutation. This Ras-independent monomer mutant is insensitive to feedback resulting in a low but constitutive Ras activity. The study suggested to overcome drug resistance through a drug combinational approach using specific RAF and MEK inhibitors resulting in a hindrance of the observed stromal regulated resistance [108].

2.1. Therapeutic strategies targeting the cancer microenvironment

Numerous complex preclinical engineering models [109] and strategies have been developed for the treatment of cancer. The impaired, leaky, vascular system and poor lymphatic drainage of cancerous tissues culminates in an enhanced permeation and retention effect known as the EPR effect [110]. Consequently, cancer drugs coupled to molecules within a certain size range, including macromolecular drugs, liposomes and nanoparticles, are passively targeted to the cancerous tissues [20,111–113]. Alternatively, markers on the cancerous cells and surrounding vascular tissues are actively targeted through the exploitation of antigen–antibody or ligand–receptor interactions [112–116]. To this end, investigators are actively developing in vitro biomimetic platforms/systems [109,117,118]. The growing knowledge of the signaling between the microenvironment and cancer cells has resulted in a new therapeutic modality: combinatorial treatments of standard drugs with drugs targeting the microenvironment should be more specific and efficient [6,15]. Different entities of the cancer microenvironment may be targeted including signaling molecules, such as Hedgehog factor (Hh) [119] and vascular endothelial cells growth factor (VEGF), as well as specific extracellular matrix proteins and integrins. Cell adhesion-mediating integrins and their associated proteins are involved in key signal transduction pathways related to proliferation and survival and may therefore be promising drug targets [120] (Fig. 2B). Integrins have been shown to regulate CAM-DR [105]. In studies of blood cancer, it seemed that leukemic CAM-DR is the result of an integrin-regulated post-transcriptional change, which is caused by degradation of activators of apoptosis such as Bim [121]. Other studies have suggested that hematologic CAM-DR is the result of an increased stability of suppressors of apoptosis and cell cycle regulators [122] and can therefore be directly manipulated.

Studies correlating integrin expression levels in human tumors with pathological outcomes, such as patient survival and metastasis, have identified several integrins that might have an important role in cancer progression. Members of the integrin β_1 family are up-regulated in invasive breast cancer cells where high levels of β_1-integrin in patient tissue have been correlated to decreased survival [123]. In vitro studies revealed the importance of β_1-integrin for the growth of cancer cells in a 3D Matrigel™ culture while conversely not affecting normal cells [124]. Another report highlighted the importance of $\alpha_3\beta_1$-integrin in pulmonary metastasis [125], while, in a multiple myeloma animal model, treatment with a $\alpha_4\beta_1$-integrin blocking antibody effectively disrupted tumor–stromal interactions decreasing tumor burden and increasing apoptosis [126]. In another more recent example, investigators observed similar responses when inhibiting tumor–stromal interactions with cilengitide (EMD 121974), a $\alpha_V\beta_3/\alpha_V\beta_5$ dual integrin inhibitor, compared to docetaxel as the second line treatment in non-small cell lung cancer [127]. This trial concluded that there are fewer adverse responses to cilengitide suggesting that these types of drugs could be used in combinatorial drug trials [127]. Furthermore, the combinatorial action of a β_1-inhibiting antibody with ionizing radiation was much more effective than either of these treatments alone [14]. After years of preclinical studies, there are now several integrin targeting drugs in clinical trials [6,8,127]. A particularly promising strategy is based on the interference of integrins expressed during neovascularization with the aim of suppressing tumor-induced stromal angiogenesis. Some trials have been concluded using etaracizumab (also known as Vitaxin, Abegrin or MEDI-522), an antibody against the vitronectin receptor $\alpha_v\beta_3$-integrin [128–131].

Alternatively, the cancer-specific environment (e.g., vascular system) has also been exploited for the targeted delivery of drug carriers [20,112,116,132] (Fig. 2B), enhancing the efficiency and reducing side effects of drugs. For example, one strategy for the delivery to the tumor tissue is to use a specific gene coupled to a cationic nanoparticle which in turn is coupled to an $\alpha_v\beta_3$-integrin targeting ligand [133]. A pre-clinical study in mice showed a 15-fold increase in drug efficiency when this method was used to deliver doxorubicin-loaded nanoparticles to integrin $\alpha_v\beta_3$ positive tumor vasculature [9].

The ultimate goal of microenvironment-targeted treatment would be to enable personalized therapy adapted both to the molecular characteristics of the specific cancer and its microenvironment [84]. Personalized treatment, considering both the patient genotype and the stromal phenotype [23,50,76,134–136], is believed to be a solution for improved treatment of this heterogeneous disease. To reach this goal, predictive markers that can be used to determine the optimal treatment for a patient are currently being verified [137,138]. An important building block in personalized treatment will be relevant biological models that enable the testing of the predictive markers such as tumor grafts in animal models [139]. With the development of miniaturized systems with high physiological relevance, a more pragmatic means of testing patient samples will be made available.

3. Microfabricated model systems for cancer research—possibilities and challenges

3.1. Advantages and disadvantages of microfabricated model system for cancer research

This section describes how microfabricated model systems can be used for cell assays in cancer research. The general advantages and disadvantages of replacing common experimental platforms with microfabricated counterparts are discussed for both open and closed systems. Open systems are defined as microfabricated substrates, for example, 2D adhesive patterns (e.g., stamps), immersed in cell culture media (Fig. 3A), while closed systems consist of microfluidics systems that enable tight control of the culture environment in three dimensions as the liquid volume is controlled and exchanged through inlet and outlet holes (Fig. 3B) [140].

3.1.1. Open systems

Initial research on controlled cell culture platforms focused on the growth of single cells, or cell colonies, on protein patterns of controlled shape and biochemical composition [55]. Thereby the role of cell shape, cell–cell interactions, cell–matrix interaction and even mechanical activation by stretching of the substrates can be specifically assessed. Throughout the years, a large set of technologies to pattern cell culture substrates with adhesive and inert areas in two dimensions have been developed. These technologies typically either rely directly on photolithographic methods or on the cheaper and versatile techniques of soft lithography [141,142]. The spotting of protein arrays is a complementary technique that can be applied when larger islands are acceptable, i.e., for the patterning of cell colonies. Methods for patterning with high reproducibility and stability over large areas have enabled the industrial production of such platforms [143].

In the booming area of 3D cell culture, microfabricated systems can provide advantages over other types of scaffolds by enabling greater control of, e.g., cell positioning and cluster size. Originally discovered in 1986, 3D Matrigel™ is a commonly used scaffold in organotypic cell culture [144]. This natural material can be tuned for higher control of the culture parameters by structuring the gel into 100 μm wide pillars by, for example, the use of soft lithography tools [145]. Seeding single cells onto these pillars reduced size polydispersity of the resultant cell colonies and eased imaging. An alternative approach molded arrays of microwells into the surface of collagen I enabling the creation of microtissues with controlled shape within a day of cell seeding [146]. A drawback of using ECM-based scaffold materials is that cells may migrate out of cavities. Undesired cell migration can be mitigated by, for example, placing a collagen lid on top of the microwell array. A successful strategy to spatially control the position of microtissues in such an array is to culture cells in microwells surrounded by non-adhesive (i.e., inert) areas. Platforms based on this principle have been extensively investigated in our research group using PDMS microwell arrays [47,51,57,58,147] and by Lütolf, et al. using a microstructured PEG-hydrogel [148], as well as by others. Such platforms provide greater flexibility in the biochemical interface presented to the cells, as the substrate used to fabricate the microwells can be coated with many different proteins. A creative example of how microfabricated 3D model system can enable new studies is the use of magnetic actuated microcantilevers to induce mechanical stress in the microenvironment of hydrogels. The groups of Chen and Reich recently published an elegant study in which they used hydrogels suspended in between an array of flexible columns to assess tissue mechanics. The study successfully separated the mechanical contributions of cell vs. matrices in either static or dynamic loading conditions [149].

One disadvantage of the open format is that it is not always possible to address the individual units in an array. Hence, even though these open cell arrays contain a high number of experimental units with controlled environmental conditions, the full multiplexing potential of the arrays cannot always be fulfilled. To overcome this problem, Lee, et al. [150] combined the spotting of drugs on a chip with subsequent stamping onto the cell array. While this approach promises to increase the multiplexing of open systems, there may be issues with the control of the final drug dose and the avoidance of cross-contamination in

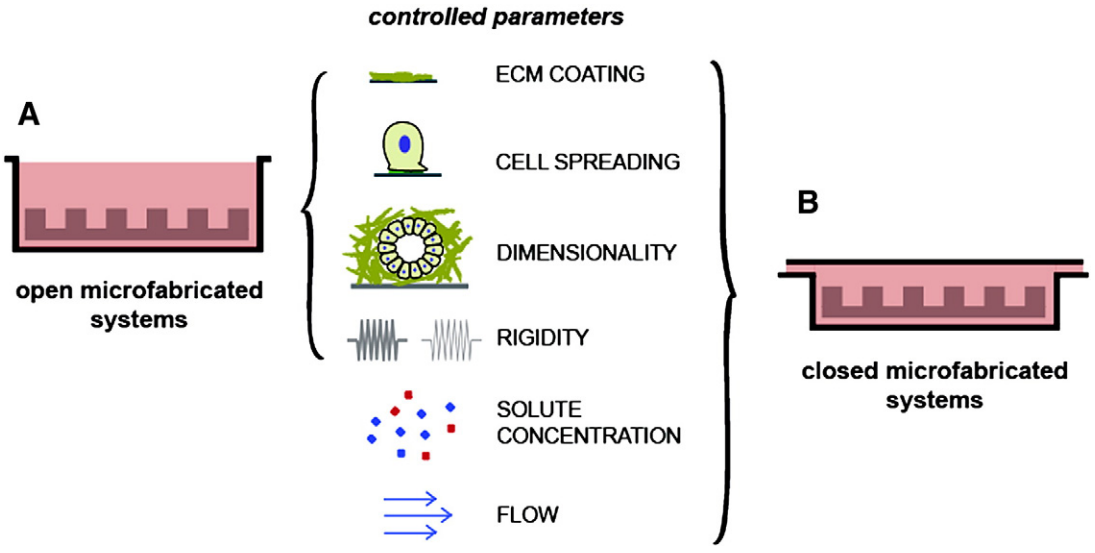

Fig. 3. Controlling microenvironmental parameters by microfabrication. Engineering on the single cell level makes it possible to control the cell microenvironment on many different levels. The controllable parameters include the ECM coating, cell spreading (cell shape), dimensionality and rigidity for an open microfabricated system (A). In a closed system (B), the liquid volume is controlled, and thereby the concentration of solutes and flow are additional parameters that can be controlled in such a system.

rinsing steps. Addressability can also be added by directly spotting samples into slightly larger microwells, for example, by using robotic dispensing to spot a large range of plasmid-DNA in a microwell array [151]. After cell seeding, the whole chip is immersed in culture media to ensure enough nutrients and inhibit drying of the sample. Hence, this strategy is only feasible because the DNA was trapped in a denatured protein matrix after spotting; otherwise cross-contamination would occur. In conclusion, multiplexing on a 2D array can be performed when molecules, such as DNA or proteins, can be reliably fixed in the spots. However, the multiplexing of cells or small molecules on these platforms requires further engineering, which could, for example, include the embedding of small drugs into disks of a biodegradable polymer for slow and local diffusion into the immediately adjacent cell culture [152].

3.1.2. Closed systems

Closed systems have the advantage of enabling complete control of the culture environment by means of microfluidic engineering (Fig. 3B). Such systems can be used for cell capture and growth, as well as to perform cell-based assays. A clear advantage is the control of the liquid flow at in vivo relevant (capillary) dimensions, which makes it possible to regulate nutrient and drug concentrations at the levels of single cells or small cell clusters [153]. In particular, it is possible to investigate pharmacological responses with very high spatial and temporal control of local drug concentration [154] enabling experiments not possible in bulk. Laminar flow regimes can easily be created in a microchannel and are characterized by low turbulence and mixing of parallel streams by diffusion only. This effect can be utilized both for the loading of cells into wells [155] and for the creation of well-defined concentration gradients within a given channel [156]. In contrast to open systems, the closed channels make it possible to individually address cells positioned in an array without cross-contamination. This can be obtained by placing several channels in parallel over the substrate or by using laminar flow. The sequential delivery of soluble factors to cells in a complex network of microchannels can be automatically controlled using electric or flow-controlled valves [157].

There is a growing awareness of the difference in cell behavior in 2D vs. 3D microenvironments; for example, cell migration differs depending on the dimensionality of the model system [28,158,159]. Thus, it is important to develop 3D environments within channels, for example, by filling microchannels with different gel forming materials such as Matrigel™, collagen or a synthetic peptide-based materials [160,161]. Thereby the advantages of 3D culture can be combined with the closed system characteristics that allow tight management of soluble factor concentration (i.e., cytokines, chemokines, growth factors, oxygen, levels of acidity and others) as well as controlled flow rates.

In the initial characterization of such systems, Kim, et al. demonstrated that cancer cells within a protein 3D gel inside a microchannel had a higher viability compared to cells grown in the gel under static conditions [63]. This discrepancy may be explained by improved in vivo-like conditions such as increased mass transfer and/or physiological solute concentrations in the channel for which the flow and metabolite concentration characteristics can be closely modeled [160]. This is important, as even very low flow rates can dramatically influence cellular behaviors (e.g., migration) [162,163], which can make it difficult to decouple the influence of solute gradients and flow. This is disadvantageous, for example, in the study of the effect of interstitial flow, which is typically reduced in cancer. Using a smart gel-based approach to enable decoupling, Haessler, et al. showed that an agarose gel could serve as a physical barrier for convective flow between channels and thereby provide a model for the study of flow-independent 3D chemotaxis [164].

The 3D microfluidic approach also makes it possible to mimic the fluid exchange between capillaries and the interstitial space, which comprises an important determinant of drug delivery and uptake in tissue [20]. Using a hydrogel-filled system, the effect of reduced interstitial flow on particle uptake with high time resolution could be studied [165]. The same principle can be used, in a simplistic manner, to mimic oxygen and nutrient uptake in transformed tissue [166].

The combination of an encapsulated compliant material with flow comes with a number of practical difficulties including how to perform cell loading, mass transfer limitations and added operational complexity. To circumvent the issue of cell loading, cancer cells may be cultured in the channel on top of a 3D Matrigel™ coating [65]. Another alternative is to produce 3D multi-cellular structures produced in a channel where the gel is replaced by a rigid pillar array [167]. Pillars and gels with higher flow resistance can also be used to stabilize more fragile gels like collagen I [162]. 3D culture in channels can also be achieved by capturing multiple cells within cell traps to form cell clusters or spheroids. Wu, et al. used an array of U-shaped traps to enable drug discovery screens on cancer cell clusters with a very narrow size distribution [155], which is difficult to achieve by other methods.

3.2. Technical challenges and opportunities

Introducing new technologies in biosciences is a difficult task, primarily because experimentation in this field usually relies on well-established methods. Even though a technology may seem highly promising, the hurdle of calibration and comparability to the huge database of results from standard screens can prevent the transition of the product from the lab to more wide usage. It is therefore important to involve institutions focusing on drug discovery at an early stage of the design process to ensure the development of useful tools and enable validation of novel platforms by comparison with current methods. In this section we review challenges, which must be overcome before miniaturized methods may find widespread use. Firstly, the use of microfabricated systems must be simplified [168]. Secondly, depending on the function of the system, it may need to be compatible with automated experimentation. Conversely, miniaturization has the power to not only improve standard analytical technologies by reducing the signal to noise ratio and by integration of several steps to reduce the time needed for an experiment but also minimize experimental errors such as sample dilution. In this section, we also discuss the possibility for improvement and innovation of analytical techniques based on the unique physical principles in miniaturized systems.

3.2.1. Adaptation to automatic handling systems and standard read-outs

New cell analysis methods can find wider use, provided they can be integrated into already established experimental infrastructure. This includes the adaptation of the new methods to standard well plate formats that can then be handled in automated systems for liquid exchange, cell seeding and assay analysis. For example, Meyvantsson, et al. elegantly integrated their passive pumping microchannels for controlled cell culture into a 96-well plate format [169]. The same group later showed that this technology could be used to perform high content chemotaxis experiments with a 50-fold increase in throughput compared to conventional assays [170]. In general, open microfabricated systems are more easily adapted for automation setups than closed systems. Closed systems still suffer from a difficult loading process, as well as the dependence on bulky pumping units. On the other hand, all types of 2D and 3D cell arrays can be easily monitored by robotic systems [140,143,171,172], thereby enabling at least medium-throughput screens.

Adapting standard assay read-outs to microfabricated model systems is another means of increasing the use of these systems in cell biology and drug development research. Today, solution-based extracellular assays measuring, e.g., cell metabolites are still standard in high-throughput screening [173]. However, in the last decade image-based read-outs have been increasingly used [174] because they provide data relating to the sub-cellular level and thereby enable more detailed information about the treatment response in a given cell population. In high content screening, several signals related to cell behavior are collected to produce a matrix of information about each sample [175]. For

example, measuring a number of different markers of apoptosis, e.g., nuclear fragmentation, caspase-3 activity and mitochondrial activity [176], yields a more robust output than any single parameter would. Lately, high content screening methods have been adapted to real time imaging of living cells recording effects of drugs upon cell cycle or migration [177,178].

In contrast to animal models, as well as many standard in vitro systems, the cellular events in a microfabricated system can be studied by high-resolution imaging with high temporal accuracy. Even when 3D cell culture is performed within a microfabricated system, the small dimensions allow the whole sample depth to fit within the focus depth of a normal objective. There are several transparent materials that can be microfabricated, such as polystyrene [179], PDMS [180] and some hydrogels [148]. Even though PDMS is known to be relatively affordable, inert and ideal for rapid prototyping, it is a non-standard material in biological research. Therefore, rapid prototyping of thermoplastics such as polystyrene [181] and cyclic olefin polymer [182] may become very important. These methods require some additional equipment compared to soft-lithography, such as a hot press and more stable molds, but will enable the researcher to develop processes that can be transferred directly to industrialization.

Standard solution-based assays [183] can easily be adapted to the miniaturized format. The smaller volumes require fewer cells per sample. However, it is important to consider artifacts on the signal coming from surface adsorption of molecules, errors in dilutions and cell populations below a critical cell number. If such factors are taken care of by choosing appropriate material and coating, using a well-designed device and by calibrating the read-out, it is possible to assure a high signal to noise ratio. On the other hand, intracellular staining assays, such as Annexin V-PE [184] can be cell number-independent and used to measure cell response also in single cell samples. This type of Cytometry gives information of the distribution of cell response within a population. High content screening procedures require high-resolution imaging, but can be adapted to microfluidic systems if the material in the imaging area is clear and suitably thin [185].

The quantitative analysis of cells present in a 3D environment is in general challenging. Currently, typically two different approaches are utilized with satisfying results; microarray scanners to measure the integrated intensity in a 3D cell sample [150] or high-resolution confocal microscopy to determine, e.g., cell death in a high-content analysis fashion on the single cell level [62].

3.2.2. Microfabrication can improve conventional assays and lead to innovation of analytical techniques

There are numerous examples of how microfabrication can be used to improve existing assays, e.g., in morphology-based readouts. Using etched 3D microstructures, transformed cells could be more easily distinguished from control cells [186]. Another group showed that, as a consequence of growing cells on single-cell patterns, the signal to noise ratio is improved due to a reduced variability in intracellular organization [187]. A recent report shows how microfabrication can be used to improve the standard comet-assay, which determines the electric field-promoted travel distance of DNA fragments in an agarose gel as a measure of DNA fragmentation due to apoptosis. The new platform showed that, by microfabrication of the agarose gel, single or multiple 3D cell culture, drug exposure and DNA analysis could be combined on one substrate [188].

With a focus on the advantages of these methods and with the broadening interfacing between engineers and biologists, we could expect that many more standard methods may be simplified by microfabrication. Clearly, the miniaturization efforts will also contribute to the development of completely new solutions for the analysis of cell behavior. A direct consequence of miniaturization is the possibility of performing single cell analysis on both adherent cells [147] and cells in suspension [189]. Single cell analyses of cells in suspension assessing individual cell mechanics, such as stretchability, have been used in a high throughput manner to differentiate between invasive and non-invasive cells [190]. By studying the cell migration through narrow constrictions in a microfabricated chip [191], the distinction between different cell types in a heterogeneous cell population is made possible [192]. Such a system could be developed towards a diagnostic tool or used as a functional read-out when assaying invasion-inhibiting drugs.

Hence, it will not be long before researchers develop methodologies for single cell drug responses using these methods (e.g., for circulating tumor cell assessments and predictions). Moreover, for adherent cells, single cell analysis on chip, with organized cell positioning in x–y–z, enables serial and time-dependent analyses of, e.g., different steps of apoptosis within a population [193]. Microfabrication also offers alternatives to microscopy-based single cell analysis by integration of chemical [194] or electrical current [195] sensor arrays placed on detection chips for fast and accurate analysis of drug response.

4. The application of microfabricated model systems for cell-based cancer drug discovery

The promise of microscale systems for improvement of cell-based assays in cancer research has led to intensive research within this field. Recent studies of novel systems usually show a relevant experimental design, in which the choice of cell type and drug or microenvironment is strongly based on the current state-of-the-art in biological in vitro and in vivo research. However, since most studies are still used to show feasibility of a method, cancer cell lines are often used for simplicity. This may be the correct choice and follow the state-of-the art for some models, while a primary cell would have generated more relevant results in other systems. Therefore, a more thorough evaluation of many of the cited studies in this section would be needed to really show their respective predictive strengths for a certain disease types. Frequently studied cancers in these systems are the breast, prostate and colon.

4.1. 2D and 3D cell arrays to determine the effect of specific environmental parameters on cancer behavior and drug response

Unique aspects of cancer cell behavior and especially its dependence on the microenvironment have been discovered by the application of 2D cell arrays. In a pioneering study, the spreading area of cells on adhesive micropatterns was used to study cell growth vs. apoptosis [59]. Latter work also determined that cell–cell adhesion promoted cell division in normal cells but only when cells were sufficiently spread [196]. This result may explain some of the diverging responses to anti-mitotic-drugs in tissues with different cell densities. Interestingly, using a microwell array of cancer cell clusters, Håkanson, et al., recently showed that this mechanism was highly conserved in early stages of breast cancer and was directly related to E-cadherin expression [62]. In fact, the formation of cell–cell contacts via E-cadherin also affects the secretion of vascular endothelial growth factor and interleukin-8, indicating the importance of cell interactions in regulating the angiogenic potential of tumor cells [197]. Adhesive micropatterns have also been used to determine the importance of cell size and tissue geometry for the epithelial to mesenchymal transition [198,199], also known as EMT, which is critical in metastasis. Furthermore, the cell shape and the distribution of the adhesion environment impacted on intracellular contractility and mitotic spindle orientation [200,201], which has also been postulated to contribute to cancer metastasis [202]. This work has now been extended into a 3D environment to reveal that cell shape is critical in controlling mitosis, regardless of the dimensionality of the microenvironment [57]. The comparison between cells cultured in square microwells vs. 2D square patterns demonstrated that dimensionality alone also affected the progression of the cell through mitosis and spindle orientation.

3D cell arrays are favorable because of their relevance to cell behavior in vivo. Consequently, there has been a shift in recent years towards

3D models that simulate physiological settings. Controlling extrinsic parameters in an in vivo relevant environment can be used to give insight into the microenvironmental control of cell behavior. To this end, our group has proposed to use multi-well configurations of cell-derived ECMs taking advantage of the different types of ECMs produced by diverse stromal cells (e.g., normal vs. tumor-associated fibroblasts) to study matrix induced drug effects as well as cell responses to the specific ECMs in a variety of cancers [12,13,33,203]. On the other hand, others have shown that microwell arrays produced in collagen I enable the study of the role of the 3D microenvironment on various cell behaviors such as branching [204], which is relevant for the control of organogenesis, angiogenesis, invasion, and metastasis. It was shown that cell branching was affected by the local geometry of the tissue and by the biochemical signaling imparted by the surrounding ECM [43]. In fact, it has been recently proposed that local changes in tissue morphogenesis are regulated by the ECM [205]. Alternatively, by producing a microwell array using inert materials, we were able to independently vary extrinsic parameters, such as ECM composition, cell–cell adhesion, stiffness as well as the dimensionality of the culture environment [47,51,57,206]. In fact, we used a PEG-hydrogel microwell platform [15] to dissect the effect of the above-mentioned parameters on drug responses of multilayer cancer cell clusters [62] (Fig. 4A). In this recent study, we demonstrated that the two ECM proteins, laminin and collagen I, had discrete effects on breast cancer clusters treated with Taxol even under control of other factors such as cluster geometry and cell density. Optical sectioning of the clusters by confocal imaging revealed that Taxol response also depends on the prevalence of cell–cell vs. cell–matrix interactions [62].

4.2. Controlled co-culture to separate heterogeneous cell interaction from environmental factors in cancer invasion scenarios

Homogeneous cell–cell interactions between cancer cells as well as heterogeneous interactions between cancer cells and other cell types in the microenvironment are known to be crucial for the progression of cancer and metastasis [72]. Understanding the mechanisms behind cancer cell migration (and invasion) and the role of the microenvironment is critical for the development of metastasis-inhibiting cancer therapy. The importance of homo- and heterogeneous cellular interactions in normal and cancerous cells has been studied in vitro using (among other strategies) engineered cell–cell adherent receptor (e.g., cadherin) surface structures [51,207–209], as well as in co-

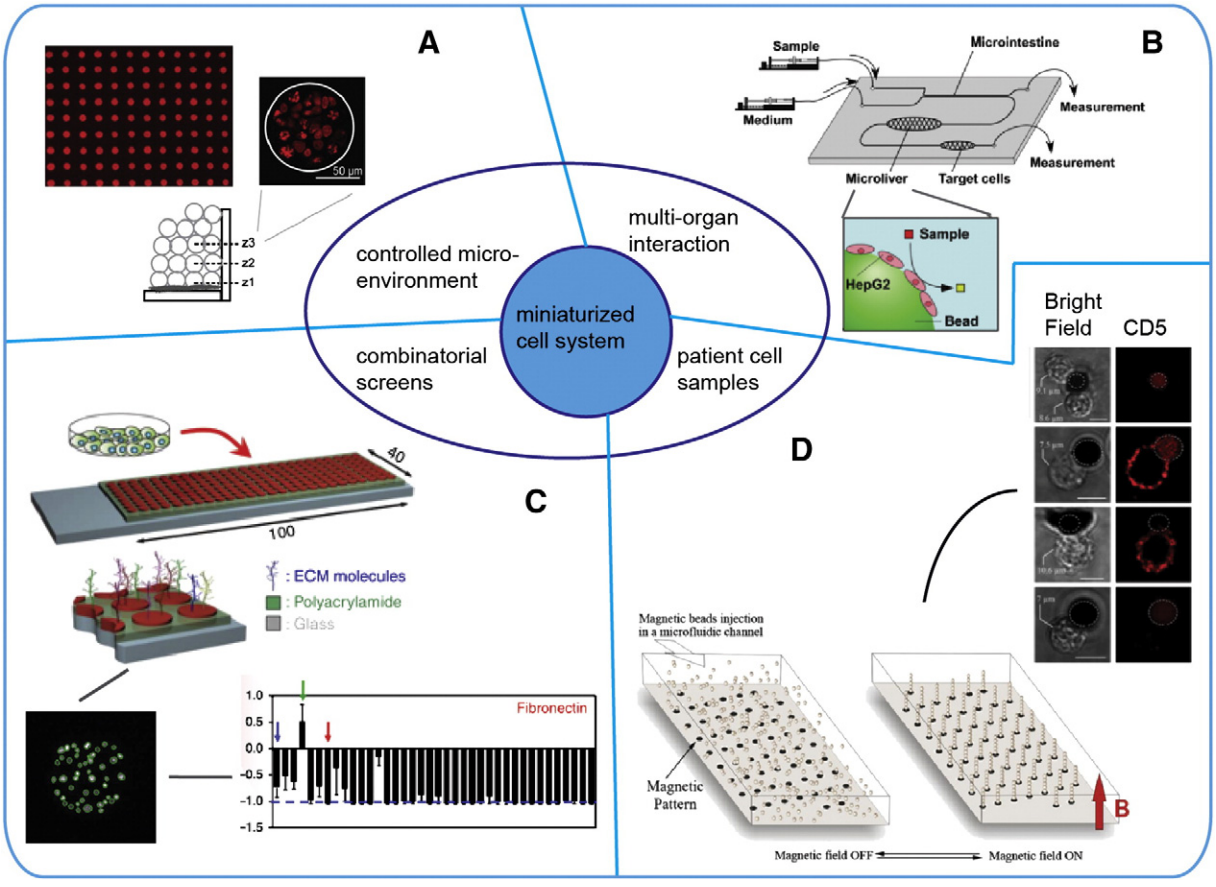

Fig. 4. The application of miniaturized cell systems in cancer drug development. Different types of cell arrays can be useful to determine the effect and the interdependence of environmental parameters on drug response. (A) A PEG-hydrogel microwell array platform with protein only at the bottom of microwells is exploited to study the effect of cell–matrix interaction and cell–cell interaction in 3D cultured breast cancer cells. On this platform, the extrinsic parameters could be controlled to mimic the niche of a breast cancer. Further, confocal microscopy allowed insight into the role of cell positioning within the cluster and cell-density, comparing the images from different positions within the clusters (z1, z2 and z3). One result of this work was that not only cell–cell interactions are important in 3D drug response but also matrix-interaction, although its effect is spatially limited [62]. Multi-organ interaction can be studied on "body-on-a-chip" platforms (B), which represent an in vitro alternative to the animal or even patient customized human model, which can test the uptake, distribution and metabolic digestion of a drug in different organs. In one approach, a system was developed to integrate the effect of the intestinal absorption and the hepatic metabolism of drugs against breast cancer [221]. Miniaturized spotted adhesion arrays (C), with mixtures of different proteins, enables the combinatorial effect of different matrix proteins to be studied. By screening different combinations of fibronectin with other matrix proteins, it was found that the adhesion of one cell line was increased on fibronectin combined with collagen IV. The same study used the array to correlate ECM ligands to metastatic behavior [171]. Miniaturization enables the immediate capture and analysis of small cell samples from patients (D). The limited number of cells in patient samples has so far restricted their use in diagnosis and treatment prediction. However, the direct analysis of patient cells is an important aspect in enabling personalized treatment. In miniaturized systems, small cell numbers can be analyzed with a signal-to-noise ratio, which is similar to that in standard macro scale analysis of larger cell populations. In a versatile approach using self-assembly of magnetic beads decorated with antibodies, cells could not only be captured within a microsystem but also further cultured therein and analyzed [242].

culture systems [210]. Tightly controlled co-cultures, in terms of the dimensions of colony islands and cell contacts, can be produced by microfabrication [211]. The possibility to mimic complex in vivo environments makes microfabrication ideally suited to study essential stages of cancer progression that rely on a complex communication with other cells, such as capillary morphogenesis and cancer cell metastasis [212]. For example, Sarkar, et al. developed a simple breast cancer system with cells present as single cells and colonies in an array surrounded by fibroblasts to study the role of homo- and heterotypic cell-adhesion in cell migration [213]. Chung, et al. used a system in which cells can migrate within a collagen I scaffold to investigate in detail the effect of cancer cells on the migration of endothelial cells, the first stage of capillary morphogenesis [214]. Rizvi, et al. recently showed how a Matrigel™-based microfluidic platform could be used to study the role of flow on the metastatic transformation of a human ovary carcinoma cell line [65].

Importantly, these platforms enable the separation of effects of, e.g., direct physical cell–cell contacts and paracrine signaling. This can provide valuable information for the assertive targeting of tumor–stromal interactions. It was, for example, shown that soluble signals from hepatocellular cancer cells increased the migratory activity of fibroblasts in a concentration- and time-dependent manner [215]. In another system, the microfluidic compartmentalization of a collagen I gel enabled the separation of factors, thereby unraveling two different mechanisms for the role of fibroblastic cells in the transition of breast cancer cells to migrative (invasive) cellular behaviors [216]. Both soluble factors and direct cancer cell to fibroblast cell contacts were shown to be important.

A multitude of parameters play a role in the mechanisms controlling cancer cell movement within the vascular system, including shear force. Using a device with a thin membrane separating two channels, Song, et al. presented a detailed study of the heterogeneous cell interactions [217]. Using an endothelial cell layer on the membrane to mimic the lining of the vessel wall, while metastatic tumor cells were perfused in the upper channel, the authors showed that the basal stimulation of the endothelium with the cytokine CXCL12 (also known as SDF-1) to mimic inflammation, significantly increased the adhesion of the cancer cells through their receptor CXCR4 [217]. In a recent report, a microfluidic device was used to study the role of CXCL12 together with the heterotypic interaction between salivary gland cancer and mesenchymal stem cells (MSCs) [218]. By 2D and 3D assays, precise cell patterning, stable chemokine gradient formation, and real-time evaluation of cell migration, some details of the heterotypic cell–cell interactions were accurately and quantitatively investigated. MSCs were observed to be recruited by neoplastic cells. Particularly, MSCs exhibited the ability to enhance the invasion of cancer cells under a chemokine CXCL12 gradient indicating the involvement of CXCL12–CXCR4 pathway and the role of MSCs in cancer progression [218].

The possibility to mimic the complex environment in tumor cell intravasation was also explored by Kamm and colleagues [219]. They developed a microfluidic-based system that recreates the tumor-vascular interface in three-dimensions allowing for high resolution, real-time imaging, and precise quantification of endothelial barrier function. In their device, the combined effect of biochemical factors from the participating cells and cellular interactions with macrophages were studied in three dimensions. With this method, they elegantly showed that signaling with macrophages via secretion of tumor necrosis factor alpha results in endothelial barrier impairment and increased intravasation rates. Their results provide evidence that the endothelium poses a barrier to tumor cell intravasation that can be regulated by factors present in the tumor microenvironment.

In summary, these examples show how microfabricated systems can be used to recreate and control discrete and in vivo-relevant conditions. Using such platforms to study key cell behavior in cancer may guide the way we think about tumor–stromal interactions with the goal of developing novel therapeutic approaches.

4.3. Integrated miniaturized multi-cell type systems to study cancer drug uptake and metabolism

An interesting and exciting application of closed systems is the microscale cell culture analog (μCCA), which consists of a physical representation of the physiologically based pharmacokinetic model [220]. The idea of these models, also termed "body-on-a-chip," is to present an in vitro alternative to animal or even to patient-customized human models, which can test the uptake, distribution and metabolic digestion of a drug in different organs. For this, Sung, et al. combined 3D culture with controlled flow characteristics to create an in vivo relevant organization of different tissues for pharmacokinetic and pharmacodynamic studies. This was done in a closed flow circuit consisting of several parallel culture chambers for different cell types (cell lines derived from colon neoplasia, liver and myeloblasts) to represent the tumor, liver and blood components, respectively [220]. After optimization of the residence time of an injected drug in the different compartments to mimic in vivo conditions, the metabolic cleavage of a pro-drug to 5-fluorouracil was successfully mimicked. A similar approach was designed to integrate the effect of intestinal absorption and hepatic metabolism of drugs against breast cancer [221] (Fig. 4B). Here, the same system could be used to determine the effect of 4 different drugs on a breast carcinoma cell line in a short amount of time. Hence, these systems could offer a more efficient, reproducible and economic alternative to animal models. In contrast to standard systems, microfabrication enables the relevant cells to be brought into contact with the drug at physiologically relevant scales, concentrations and retention times. Novel developments using "body-on-a-chip" and similar systems have been recently reviewed [222,223].

4.4. Combinatorial screening platforms for the tight regulation of environmental factors

The use of miniaturized culture systems for combinatorial studies merges the above-mentioned advantages of controlled culture parameters with the possibility to dramatically reduce material and reagent consumption. Therefore it makes sense to move ECM adhesion arrays from a multi-well plate to the microarray format [224]. Kuschel, et al. were among the first to explore the differential adhesion pattern of very few cells onto discrete matrix protein arrays [224]. Adhesion arrays can also be used for screening matrix-dependent drug responses, which have traditionally been studied using multi-well plates [225]. The proteins may also be spotted in mixtures enabling the combinatorial effect of different matrix proteins to be studied. This technology was earlier used to study of the combinatorial effect of collagen I, -III, -IV, laminin and fibronectin on embryonic stem cell differentiation [172], while a more intricate array was recently constructed to correlate ECM ligands to metastatic behavior [171] (Fig. 4C) in cancer cell lines as well as primary and metastatic tumor-derived cells. In this work, the ECM ligands and integrins specific for metastatic cell adhesion were uncovered, while determination of cell growth was used to differentiate these from ECM proteins and integrins needed for matched primary tumor cell attachment. A similar platform was previously used to determine the role of cell-ECM as well as of cell–cell interactions for the fate of mammary progenitor cells [226]. Understanding the normal tissue regulation in, for example, the mammary tissue is a good base for an increased understanding of the emergence of a particular neoplasia.

There are several examples of how closed microfabricated systems have been used to investigate the potency enhancement of combination therapies [227]. One platform, based on an integrated drug mixer, was used to determine effective combinations of vinorelbine, paclitaxel and γ-lineolic acid [228] in a breast cancer cell model. Encouragingly, the results obtained were in agreement with what has previously been observed for the same drugs in standard combinational approaches in vitro [229] and more importantly in patients [230].

An interesting application is the combinatorial study of matrix proteins and solutes, considering the drastic effect of cellular and ECM interactions on drug response [33,101,225]. The NF-κB pathway is one of the more frequently targeted pathways in drug development. Using a diffusion-based device combined with a protein patterned substrate and studying the effects of different ECM mixtures in combination with diverse concentrations of IGF1 and TNF-α, Ying, et al. confirmed the interaction of soluble factors with matrix proteins in the activation the NF-κB pathway [231]. This initial work was performed with immortalized endothelial cells but it would be expected that the same method could be applied for the study of complex cancer cell signaling.

The epithelial to mesenchymal transition, or EMT, is important in metastasis as well as in cell plasticity and therefore highly relevant for drug development. As this transition is influenced by microenvironmental factors [232], the study of this transition would profit from the controlled environment within a miniaturized system. In a feasibility experiment using alveolar epithelial type II cells, Park, et al. demonstrated how the role of both immobilized ECM proteins (laminin and fibronectin) and soluble factors (TGF-β1) could be investigated in one single step [233]. The integrative analysis together with a high control of the culture environment may be particularly fruitful in the development of combination therapies targeting the cancer and its microenvironment.

4.5. Enabling the analysis of patient-derived cells by miniaturization

Miniaturized systems are particularly beneficial for the analysis of small patient cell samples. As a complementary platform to cell lines, primary cells can increase predictivity of the pre-clinical screen and ease target validation (Fig. 1) [39]. In addition, analyzing patient cells for predictive markers and dose-response curves are important tools in personalized treatment [138]. However, the culture of primary cells remains difficult since it is often hard to obtain a sufficiently large sample size [39] and a homogeneous cell population (although heterogeneity may be more representative of the human onset). In addition, primary cells are more sensitive to tissue-culture passaging than normal cells and can dedifferentiate or acquire new genetic and/or epigenetic changes after only a short time in culture. Miniaturized systems can help in removing some of these hurdles, since the reduced sample volume makes it possible to obtain the required cell number directly from a biopsy or with only a few culturing passages. In addition, the specific-cell capture in miniaturized channels can help to purify a small cell sample from in vivo sources (e.g. blood).

Initial progress in this field is exemplified, for example, by the study of Beske, et al. in which sample consumption was reduced 2-fold by producing inserts for standard well plates that enabled analysis of 10 cell samples per well [234]. Moving from standard to miniaturized ECM arrays for cell adhesion assays enabled applying this platform to the characterization of cancer cells from patients [224] and, more recently, to discriminate between local primary tumor cells and cells with metastatic potential [171]. In two examples of closed microfabricated systems determining drug concentration-response curves [41] and diagnosing oncogenic kinase activity [235] in primary human leukemia cells, a much reduced number of cells were needed to produce results with signal-to-noise ratios comparable to standard assays. In fact, the cell number reduction in comparison to the conventional methods was in the most extreme case 2000-fold [235], as shown in the assay development using a model cell line. In another example, an image-based read-out facilitated single cell analysis in a microfluidic device, thereby reducing the cell numbers needed per assay even more [193]. In this device, 300 hematopoietic cells were sufficient to obtain a drug-response with a similar statistical spread to that obtained using 15,000 to 30,000 cells using conventional methods. However, while the aforementioned approaches may be powerful in cancer diagnostic and devising patient-specific treatment, reducing the experiments to a limited amount of cells may not always give a good predictivity for highly heterogenic tumors, such as renal cell carcinomas [236].

An interesting application of microfabricated systems is the analysis of circulating cancer cells (CTSs) in blood and cells from fine needle aspirates. The microfluidic platform, with its high surface area, is superior to standard methods for the capture of cancer cells present in solution [237]. This is especially important for these samples, since the concentration of CTCs may be only 1–100 per ml of blood [238]. Cells may be captured inside the chip by cell size- or cell mechanical properties-dependent and/or biochemical affinity-based methods [237–239]. This technology has been refined to create a platform that can be manufactured on a commercial scale with improved cell capture rates [240]. However, despite the promising results, no microscale system for CTC-capture is currently FDA approved [238]. Therefore, it is still important to validate the new systems, for example showing correlation to standard methods such as flow cytometry. Weigum, et al. developed a device for the early detection of oral cancer based on a micro-sieve for capture of the cells and subsequent analysis by immunochemistry [241]. It was shown that the levels of EGFR expression detected in the microscale device correlated well with flow cytometry results. Recent work combined the immunophenotypic isolation with post-culturing manipulations of patient cells [242] (Fig. 4D), thereby enabling further analysis of the cells after capture, e.g., determining their drug response characteristics in a single microfluidics device. These novel tools thus open the path for multiplex analysis of patient samples, e.g., characterization of cancer cell abundance and identification of predictive markers to help in the design of personalized therapy.

5. Conclusions and outlook

Current interdisciplinary activities in the field of miniaturization have resulted in a large number of novel in vitro models for the capture, analysis and drug response evaluation of cancer cells. Much effort has been expended on technological advancement of these systems, such as novel microfluidic devices with better control of the culture environment, improving cell capture and positioning techniques, as well as in the development of innovative methods for assay read-out. What has been missing for many years is a pool of experimental data obtained in such systems, which could be compared to drug response data obtained in conventional platforms and in clinical studies. Without a large pool of examples showing the advantages of the new systems in terms of predictivity, analysis of small cell samples, etc., the overall impact of microfabricated systems on modern biology continues to be mostly theoretical and thus marginal. It is encouraging to see that the increasing collaboration between engineers and biologists has resulted in an impressive amount of data on the comparison of the new systems to the conventional multi-well plate setup. However, the very important step of comparison to clinical data, proving the improved predictivity, is still largely missing.

Here we listed several examples of how these systems can be applied to cancer research. The possibility to manipulate and control the cell environment on the microscale makes these systems advantageous in comparison to standard methods. In particular, it is possible to study the role of extrinsic parameters, which are often not easy to control using other methods. Application of these platforms has increased our understanding of the interactions between cancer cells and their microenvironment.

Many of the cell systems presented in this review were developed with the motivation to find application in the clinic and pharmaceutical industry. Still, there are only a few researchers that focus on the realization of this translation into practice. The problem is especially true for closed systems, which are typically more difficult in their handling than open ones. There is an overall consent that, in order to find wide use of these systems, adaptation to automatic handling and read-out methods is needed. Identifying which are the most promising technologies that should be adapted is a demanding step, which requires bridging the specialized fields of basic biology, bioengineering, microfluidics,

clinical research and engineering. A joint decision must be taken on key requirements of the device-producing industry and clinical practice.

Cancer drug development faces many challenges, however, the progression towards targeted and personalized treatment promises a bright future. Nonetheless, this direction requires more organotypic in vitro models and new methods for patient sample analysis. This could be a unique chance for the bioengineering community for more extensive collaborations with basic scientists and clinicians to explore the possibilities of miniaturization for cancer cell analysis. In addition to aiding in the development in environment-targeted drugs, microfabricated systems hold a great promise in the analysis of patient cell samples to detect predictive markers and determine dose-response profiles. With successful adaptation to automated handling system, microfabricated model systems may find wide applications in developing personalized diagnosis and patient-specific therapeutic modalities.

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