Bioengineering Models for Breast Cancer Research

Supplementary Issue: Targeted Therapies in Breast Cancer Treatment

Khadidiatou Guiro and Treena L. Arinzeh

Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ, USA.

ABSTRACT: Despite substantial advances in early diagnosis, breast cancer (BC) still remains a clinical challenge. Most BC models use complex in vivo models and two-dimensional monolayer cultures that do not fully mimic the tumor microenvironment. The integration of cancer biology and engineering can lead to the development of novel in vitro approaches to study BC behavior and quantitatively assess different features of the tumor microenvironment that may influence cell behavior. In this review, we present tissue engineering approaches to model BC in vitro. Recent advances in the use of three-dimensional cell culture models to study various aspects of BC disease in vitro are described. The emerging area of studying BC dormancy using these models is also reviewed.

KEYWORDS: spheroids, microfluidics, scaffolds, dormancy, three-dimensional culture, tissue engineering, tumor engineering

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CORRESPONDENCE: arinzeh@njit.edu

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Introduction

Despite significant improvements in research and development in the cancer field, about 95% of oncology drugs in clinical trials fail to receive Food and Drug Administration approval.[1] Part of the issue is the lack of suitable preclinical models that take into account the complexity of the disease and accurately represent disease progression. A growing need exists for technologies in research that can accurately recapitulate the in vivo environment. These models can represent the biological, physical, and biochemical environment of the natural extracellular matrix (ECM). Several innovations in tissue engineering have led to the design of scaffold- or matrix-based culture systems that more closely mimic the native ECM. For many years, cancer researchers have relied on two-dimensional (2D) monolayer culture studies and small animal models to study the complex tumorigenic mechanisms of angiogenesis, invasion, and metastasis. However, 2D cell culture models lack the structure for proper cell–cell and cell–matrix interactions and are not able to replicate an in vivo phenotype.[2–5] Multiple studies have used small animal models for conducting cancer research. However, there exist major differences between cancer progression in humans and animals.[3,6] Also, using animals can be very costly, laborious, and requires animal facilities as well as Institutional Animal Care and Use Committee approval.[7] Moreover, understanding specific factors such as chemical, cellular, and mechanical cues in animal models can be difficult to discern due to their complexity.[8,9] Recently, there has been promising published work on three-dimensional (3D) cell culture models developed to study breast cancer (BC) tumor progression in vitro. Results show that these models have the capability of re-establishing the cellular morphologies and phenotypes present during in vivo tumor development.[10–15] The 3D cell culture has been shown to impact cell morphology, gene/protein expression, signal transduction, proliferation, migration, polarization, and drug tolerance.[16–19] As shown in Figure 1, cancer biology combined with tissue engineering strategies (scaffolds, microfabrication, and biologically inspired culture models) enable studies of various aspects of disease dynamics across different scales.[20,21] For instance, at the tissue level, factors such as cell–cell and cell–ECM interactions, culture dimensionality, and soluble factor transport and signaling can be explored using biomaterials, scaffold fabrication techniques, and bioreactors. Moreover, at the cellular level, features such as topography and mechanical properties can be tailored using microfluidic channels.[22,23] In addition, with the integration of cancer biology and engineering, novel in vitro approaches can be used to quantitatively assess different features of the tumor microenvironment.[24–26] In this review, we discuss the recent literature using tissue engineering approaches in developing in vitro models for BC research and drug discovery. The 3D in vitro models and their applications with an emphasis on studying dormancy are described.

Three-dimensional Culture Systems

The 3D culture models are important tools in the advancement of basic cancer research. Nelson and Bissell[11] have been one of the pioneers to use 3D to model the murine mammary gland in both its normal and diseased state. They

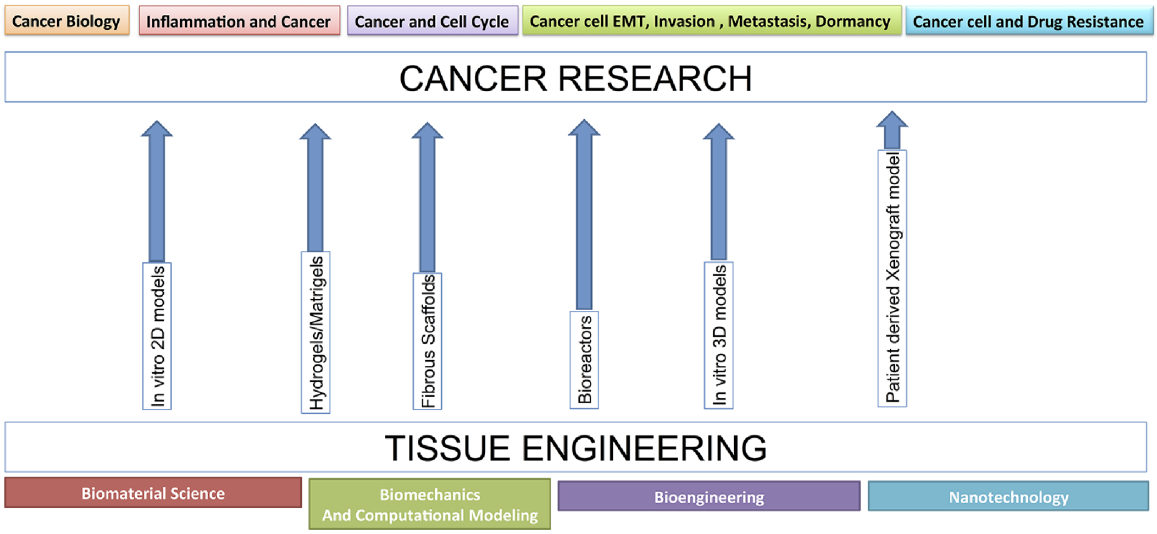

Figure 1. Tissue engineering concepts offer a powerful toolbox for cancer research areas. Modified from Hutmacher et al.[63] Reused with permission from Elsevier.

also showed that the design criteria to model BC in vitro in 3D are similar to those used in tissue engineering. Other groups have also been able to adopt their techniques to study cancer in 3D and further develop matrices to sustain organoid growth in vitro. Based on the current literature, next steps are to utilize them as predictive models in massive drug screening processes, shifting from academia to the pharmaceutical industry.

As shown in Figure 2, 3D in vitro culture systems to model tumors can be mainly categorized into cells cultured as multicellular aggregates (spheroids) and cells embedded in constructs made of natural or synthetic matrices.[27] In addition, in vitro systems have been developed to model tumors and the influence of the microenvironment using high-throughput technologies such as microfluidic devices. These systems are described in more detail in the following sections.

Spheroids. Despite advances in the treatment of BC, mortality rates of this disease still remain very high, primarily due to metastases in other organs such as bone, brain, and lungs.[1] As depicted by Talmadge,[28] these metastases start as single cell that detaches from the primary breast tumor and travel through the bloodstream or the lymphatic system to a secondary site, where at first they form micrometastases that stay undetected. Current therapies using cytotoxic drugs are delivered systemically causing serious side effects for patients and frequently do not offer prevention of long-term metastases.[29,30] Spheroids have been widely used in the cancer field as a model system in several studies involving 3D cell culture for drug

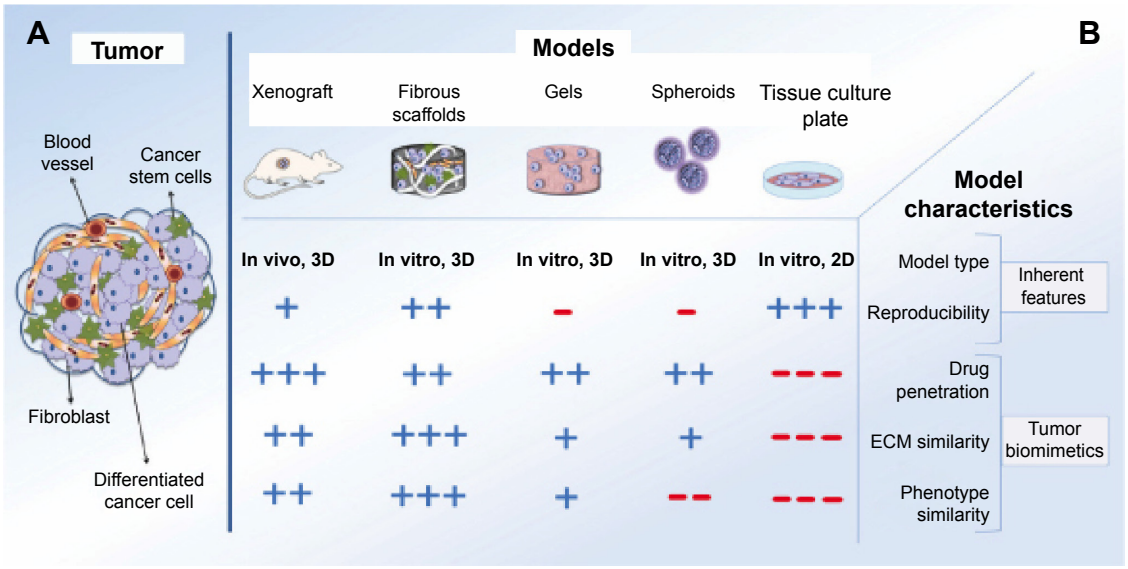

Figure 2. Schematic picture of (**A**) a tumor and (**B**) different tumor models. Modified from Ricci et al.[27] Reused under the terms of a CC-BY license.

screening predominantly for high-throughput applications (Fig. 3).[31] Spheroids are 20–1000 μm diameter clusters of cells that self-aggregate when cultured in rotary wall vessels or spinner flasks. Spheroid cultures have been described to generate heterogeneous cell populations that vary in response to diffusion limitations similar to the in vivo environment and unlike cells seeded on 2D monolayers.[32,33] Spheroids have been shown to preserve the physiological shape of the tumor, respond to chemotherapy and radiation therapy,[17,20,34] and maintain tissue-specific properties of the primary tissue.[33,35,36] Moreover, the 3D arrangements of the spheroids facilitate differentiation to support expansion of heterogeneous subpopulations similar to that observed in vivo.[18,37] Several techniques have been presented in the literature for culturing spheroids. The most widely used techniques involve growing cells on plates coated with low attachment substrates to prevent cell–substrate interaction,[38,39] in hanging drops,[31] or in a continually rotated suspension such as a spinner flask.[40] As shown in Figures 4 and 5, Markovitz-Bishitz et al[41] also were able to grow mature spheroids in microchambers as a drug screening tool. However, these techniques are time consuming and hard to standardize because they often produce spheroids with a nonuniform range of shapes and sizes with the lack of control in the cell aggregation process. To address some limitations of traditional spheroids, recent reports in the field have shown controlled 3D culture in Matrigel microbeads to analyze clonal

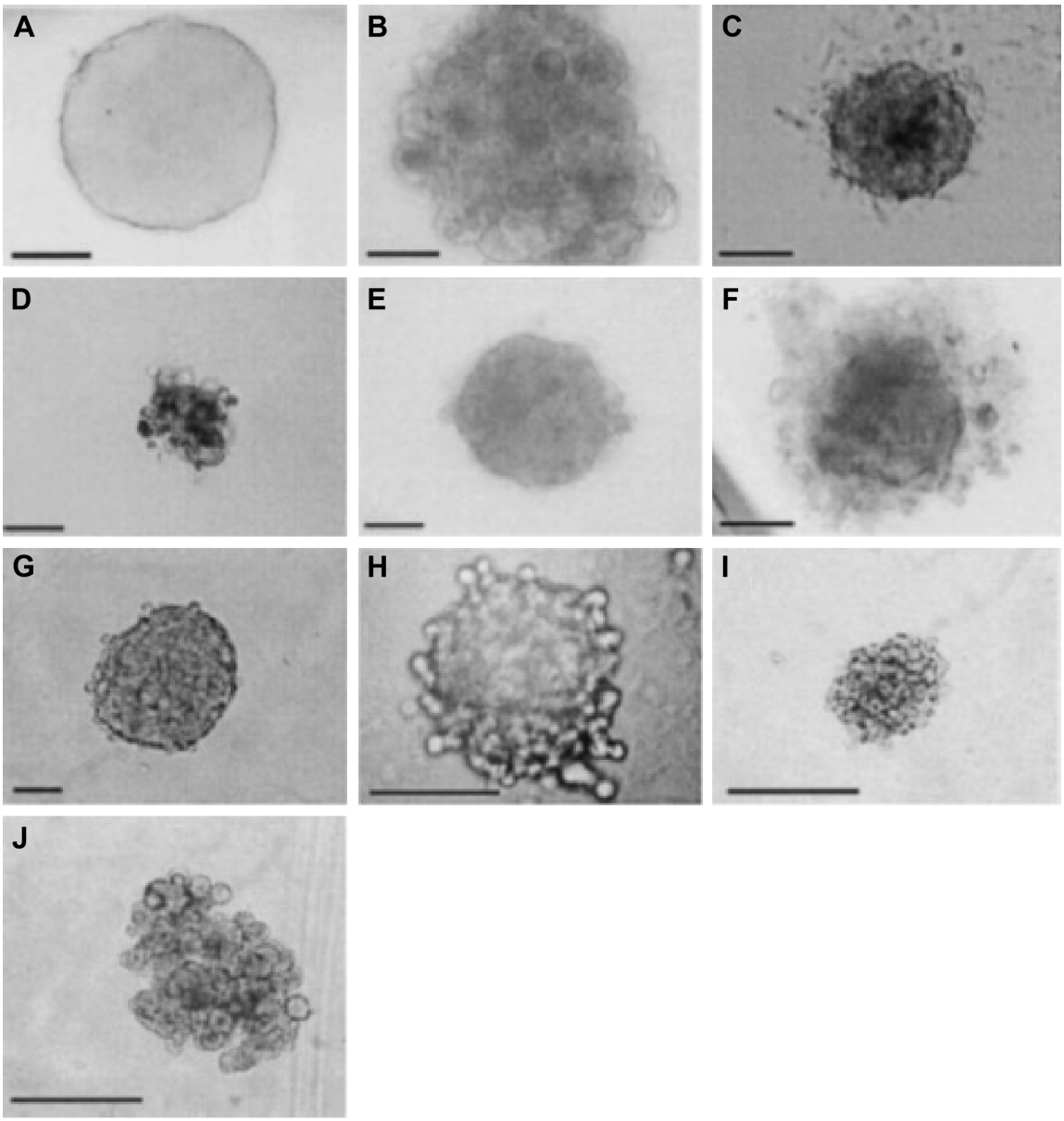

Figure 3. Spheroids of various cell lines generated by the hanging drop method (bar = 100 μm). (**A**) MCF-7 (mammary gland adenocarcinoma); (**B**) WW; (**C**) MM483 (human multiple myeloma); (**D**) MM239 (human multiple myeloma); (**E**) ME1402 (human melanoma); (**F**) MCF-10a (mammary gland fibrocystic disease – nontumorigenic); (**G**) DU-145 (human prostate carcinoma); (**H**) HT-1080 (fibrosarcoma); (**I**) HeLa (human cervical carcinoma); and (**J**) Caco-2 (human colon adenocarcinoma). Reused from Kelm JM et al[31] with permission from the publisher, John Wiley and Sons. Copyright 2003 Wiley Periodicals, Inc.

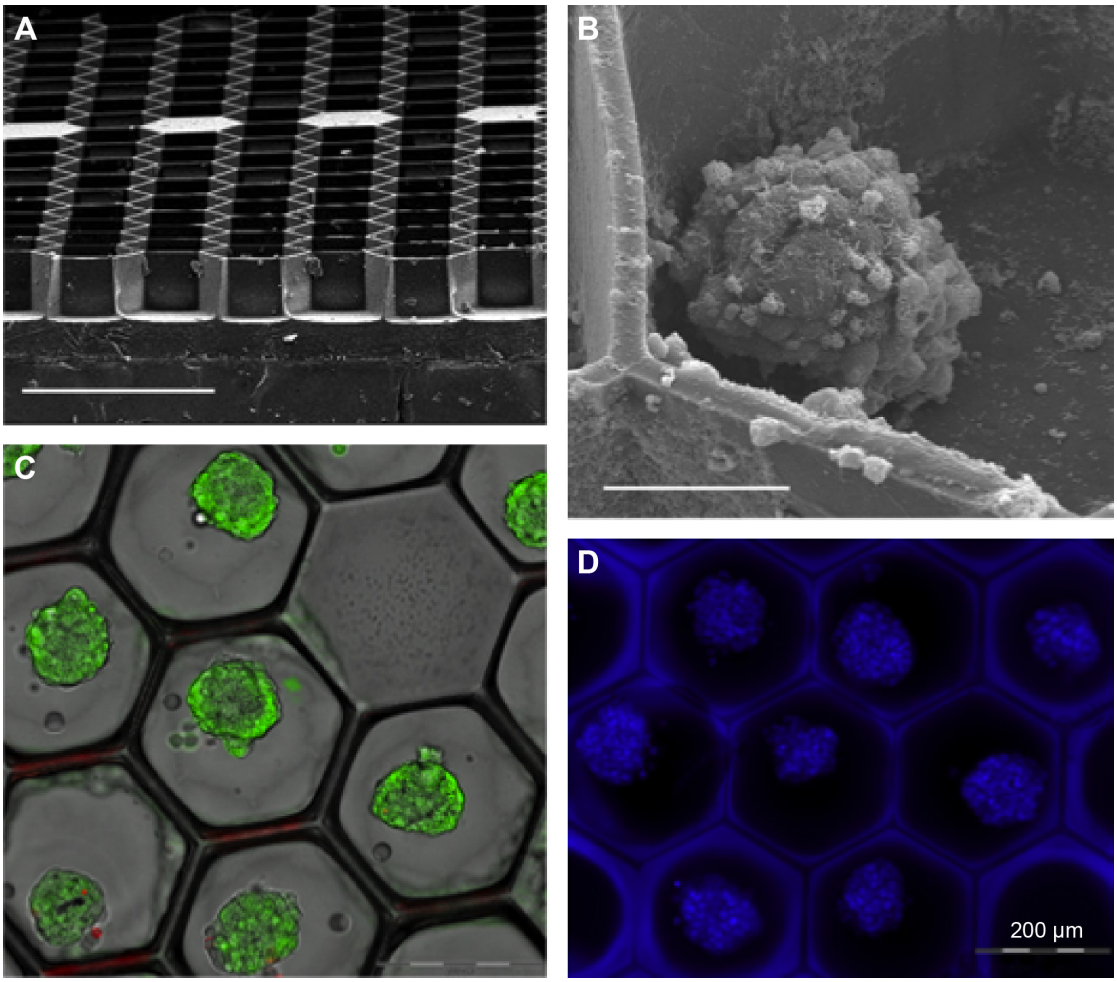

Figure 4. Spheroids cultured in microchambers. (**A**) Scanning electron microscopy (SEM) micrograph of the microstructure array embossed on a glass surface. Note the dense honeycomb structure, the sharp edges between the microchambers and the filled microchambers that form built-in points of origin on the array. Scale bar: 500 µm. (**B**) SEM micrograph of one spheroid in the microchamber. Scale bar: 50 µm. (**C**) Structured illumination images of live dead staining of spheroids after 72 hours, overlapped with transmission images. Green staining (FDA) indicates live cells, while red (PI) indicates dead cells. Note that dead cells are rarely observed. Scale bar: 200 µm. (**D**) Fluorescence image of Hoechst 33342-stained spheroids in the microchamber array. Scale bar: 200 µm. Reused from Markovitz-Bishitz Y et al,[41] with permission from Elsevier.

acinar development.[42] For instance, Dolega et al have established and optimized a technique for microfluidic Matrigel droplet formation where epithelial cells are encapsulated, so that on an average a single acinus is formed per single Matrigel bead. The group also mentions that with their approach acini culture homogeneity is conserved, and 3D structures are recapitulated for further analysis such as fundamental genomics and flow-based high-throughput analysis.[42]

Microfluidics. Recent advances in microfluidic technology have made it possible to develop innovative assays that enable accurate control of the cellular microenvironment,[43] thus addressing limitations of several assays that fail to allow user-defined microenvironments where chemical, physical, and mechanical stimuli can be accurately controlled. Microfluidic assays are highly beneficial toward clinical applications since they are high throughput and automated, thus requiring minimal manual operations during measurements.[44] Cell seeding in microfluidic systems is usually done by loading cells suspended in fluid or hydrogel solution, with cell movement being monitored after establishment of chemokine gradient or flow conditions.[45–48] For instance, Polacheck et al[48] established a microfluidic cell culture system to investigate the effects of interstitial flow on tumor cell migration (Figs. 6 and 7) and found that breast cancer cells (BCCs) migrated in an organized fashion with interstitial flow as compared to control devices without flow where cells migrated randomly. Similar to spheroid cultures, several 3D models have been established using microfluidic devices[49,50] to study metastasis initiation and progression. Some of the leading fabrication techniques have been proposed by Stroock and Fischbach[51] to address the drawbacks of conventional in vitro technologies lacking variation of soluble chemicals or a representative model of in vivo mass transport. Moreover, more recently, several investigators have applied microfabrication technologies to obtain engineered biomimetic vasculature in order to simulate physiological transport phenomena within these microfluidic

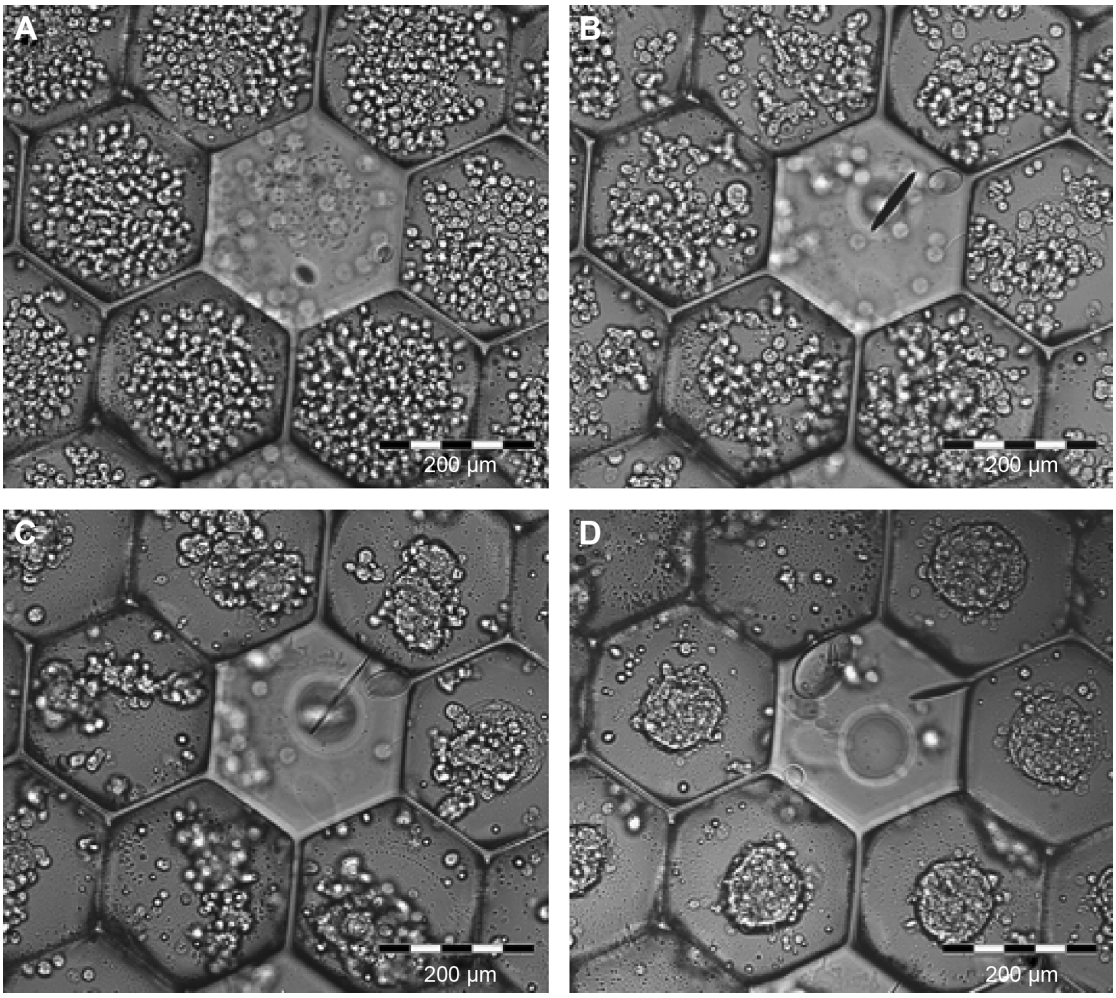

Figure 5. Assembly process of the individual cells on the microstructure. (**A**) Initial distribution immediately after seeding. (**B**) Six hours after seeding. (**C**) Eighteen hours after seeding (note that by this time most cells in each microchamber are already arranged in one amorphous cluster). (**D**) Mature spheroids, 48 hours after seeding. Reused from Markovitz-Bishitz Y et al,[41] with permission from Elsevier.

conduits.[23,52,53] The main objective of these studies is to characterize the processes activated by cancer cells under shear stress conditions: adhesion with endothelial cells (ECs) and degradation of the basement membrane to undergo metastatic growth. Furthermore, in these devices, 3D cultures made with Matrigel[54,55] or collagen[48,56–58] scaffolds are subjected to a continuous flow (shear stress, interstitial fluid flow). The main goals are to allow for the analysis of epithelial–mesenchymal transition (EMT) processes as a function of fluid forces during tumorigenesis. However, a major disadvantage of these high-throughput microfluidic assays is that the information content is too simplistic and does not fully depict the complexity of a biological phenomenon. For instance, microfluidic culture systems are constrained to very small artificial environments in the order of few hundreds of microns, which fail to mimic the heterogeneous complexity of breast metastatic niches. In addition, these small environments with relatively low seeding densities can be challenging for some biochemical assays.[38,59–62]

Scaffolds. The 3D culture techniques usually include adding cell suspension to matrices such as type I collagen or Matrigel or culturing cells on biomaterial scaffolds that can be fabricated into various desired architectures from different materials.[20,25,63–65] Hydrogel matrices, such as type I collagen and Matrigel, and synthetic matrices have been widely used to investigate how the physical properties of ECM modulates tumor cell invasion.[66,67] Generally, in these studies, tumor cells are uniformly seeded inside a homogeneous 3D ECM and their phenotypic characteristics are monitored in real time or after a given time period.[67,68] Many groups have shown the benefits of using 3D scaffolds over 2D tissue culture polystyrene (TCP) for obtaining an in vivo phenotype.[2–5,26] In general, studies comparing 3D models using biomaterials to 2D monolayer cultures using cell lines across a range of cancer types have demonstrated in vitro proliferation rates closer to those found in vivo,[10,69] increased gene expression, especially upregulation of angiogenic factors,[10,69–71] and enhanced drug resistance (Fig. 8).[5,10,69,72]

Natural biomaterials. Matrigel is a basement membrane formulation derived from the Engelbreth–Holm–Swarm mouse sarcoma. The major ECM components of Matrigel

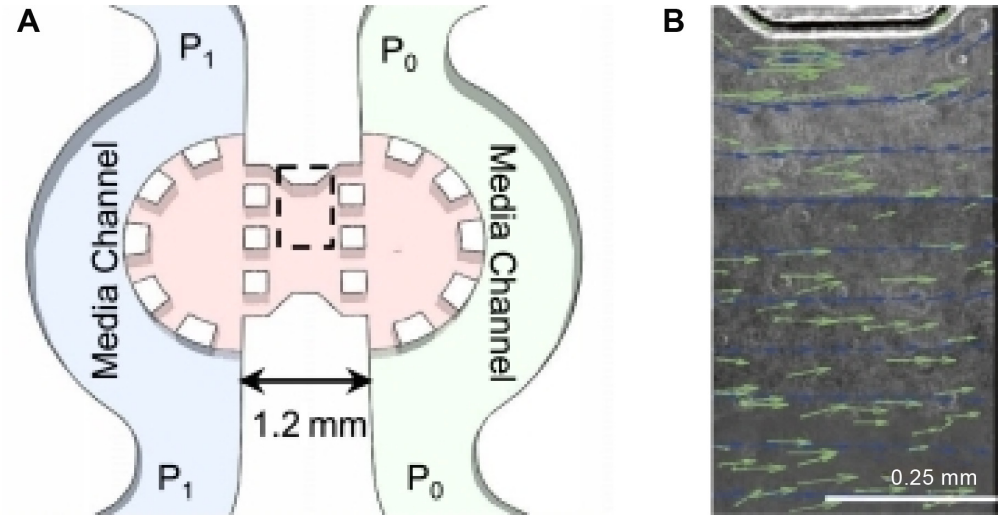

Figure 6. Microfluidic cell culture system for investigating the effects of interstitial flow on tumor cell migration. (**A**) Schematic of the microfluidic device. The device consists of two channels (P1 and P0) separated by a region in which cells are suspended in collagen type I gel. By applying a pressure gradient across the gel, a consistent flow field is generated. To validate the flow field, fluorescent microspheres were introduced into the bulk media and time-lapse images were taken to track the beads. (**B**) Velocity vectors observed by tracking the fluorescent microspheres (green) superimposed on streamline vectors for a computation model (blue) and on a composite phase contrast image of the region of the device indicated by the dashed line in A. The composite phase contrast image is comprised of subregions that were imaged sequentially to measure velocity throughout the whole gel region. Reused from Polacheck WJ et al.[48] Copyright is retained by the authors of the original work.

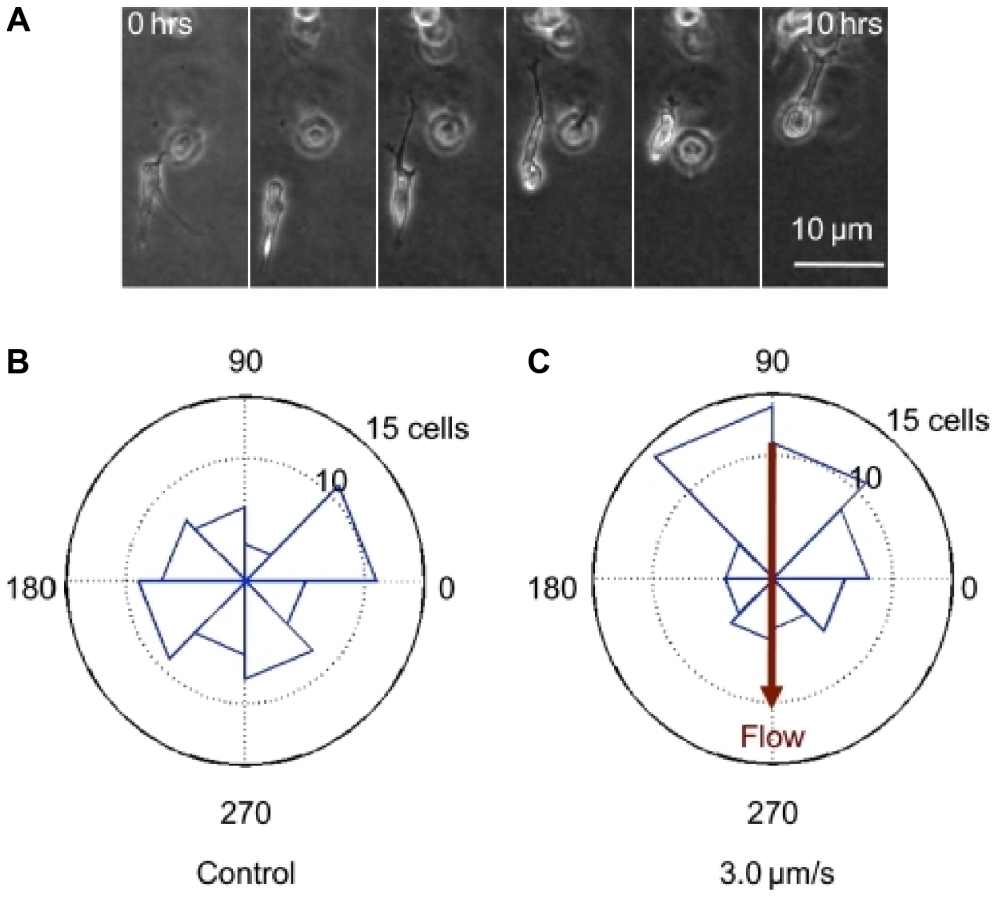

Figure 7. Interstitial flow influences direction of cell migration. (**A**) Sample time-lapse images of a cell migrating in an interstitial flow field. Flow is 3.0 μm/s from top to bottom in the image. (**B**) Sample data from one control device. The polar histogram demonstrates distribution of angles of net migration vectors for cells in a population in one device. Cells in control devices without flow migrate randomly. (**C**) Flow changes the distribution of migration vector angles. Reused from Polacheck WJ et al.[48] Copyright is retained by the authors of the original work.

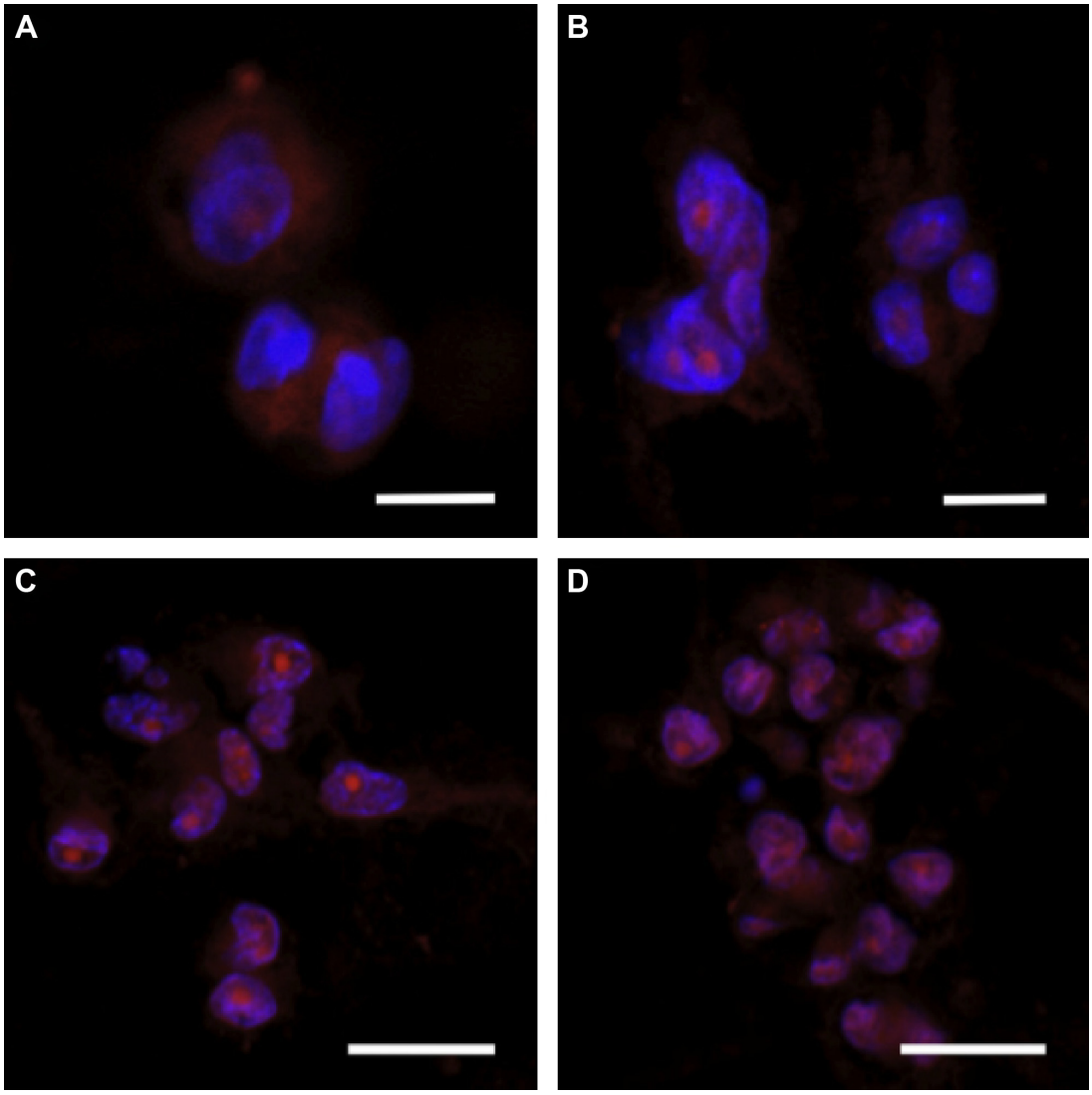

Figure 8. Breast cancer cells cultured on collagen I hydrogels. MDA-MB-231 cells were cultured in collagen I hydrogels for one, three, five, and seven days (**A**–**D**, respectively), exhibiting the typical cell–matrix and cell–cell interactions observed in vivo. Cells developed an elongated morphology over seven days with visible processes, demonstrating cell–matrix interactions. As the cells began to proliferate, they aggregated into 3D clusters, demonstrating cell–cell interactions. Scale bars are (**A**, **B**) 10 μm and (**C**, **D**) 20 μm. Reused from Szot CS et al,[71] with permission from Elsevier.

include laminin-1 (α1β1γ1), collagen IV, enactin (nidogen-1), and perlecan (heparin sulfate proteoglycan). Moreover, type I collagen is used as a substrate for cell culture and tissue engineering applications since it contains the tripeptide RGD (Arg–Gly–Asp), a short amino acid sequence that binds to receptors on cell surfaces.[73] Type I collagen hydrogels possess 3D architecture and biocompatibility that are ideal properties to mimic some of the conditions of the tumor microenvironment such as cell-mediated degradation of collagen allowing for remodeling of the matrix during proliferation, migration, and infiltration.[74] Type I collagen-based hydrogels can promote cell adhesion, proliferation, and the formation of large cell clusters.[2,26]

Matrigel and type I collagen isolated from animal tissues[75] possess unique characteristics that can be modulated by adjusting gelling conditions such as gel thickness, temperature, and concentration.[76–78] However, when using Matrigel with cells, several studies have also identified batch-to-batch variations that cannot be controlled and can be problematic when interpreting results from these studies,[79–81] suggesting that improved 3D systems are still needed.

Synthetic biomaterials. To address the shortcomings of natural biomaterials, synthetic materials have been widely used by cancer biologists and engineers. Synthetic biomaterials can be fabricated in a reproducible manner and in large quantities. Some of the commonly used synthetic biomaterials to generate BC models include poly(lactide-co-glycolide) (PLG),[10] poly(lactic acid) (PLA),[5,82] and poly(ethylene glycol).[83] Paszek et al[84] found that combining polyacrylamide gels with ECM components and changing the elastic moduli can disrupt epithelial tissue homeostasis, potentially leading to malignant behavior. Recently, Ghajar and Bissell[20] have described tumor

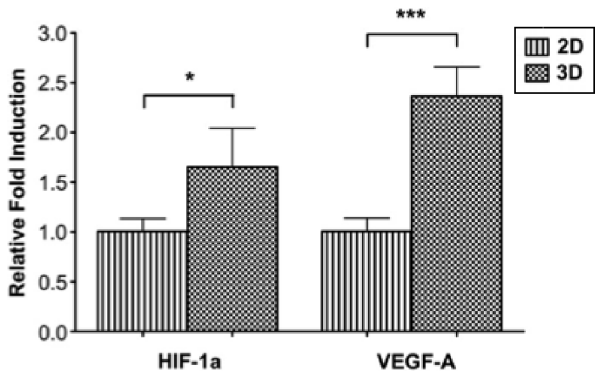

Figure 9. Hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor-A (VEGF-A) gene expression of MDA-MB-231 cells cultured in 3D type I collagen hydrogels in comparison to cells cultured on 2D tissue culture polystyrene. Gene expressions were significantly upregulated when MDA-MB-231 cells were cultured in 3D type I collagen hydrogels as compared to cells cultured in a monolayer on 2D tissue culture polystyrene. Gene expression was compared on day 0 to determine the specific effect of 3D culture without the contribution of cell proliferation or the development of hypoxia. $*P < 0.05$ and $***P < 0.001$, respectively. Reused from Szot CS et al,[71] with permission from Elsevier.

engineering as "the construction of complex *in vitro* culture models that mimic aspects of the *in vivo* tumor microenvironment to study the dynamics of tumor development, progression, and therapy happening in multiple scales." Fischbach et al,[10] Cross et al,[13] and Szot[71] have engineered 3D in vitro tumor models using both natural and synthetic polymeric scaffolds, respectively, showing that ECs can remodel dense type I collagen matrices in response to angiogenic factors from cancer cells (Fig. 9) and the different effects of these angiogenic factors on drug responsiveness. Embedding cancer cells in 3D hydrogels may induce stress, such as limited oxygen and nutrient transport, as compared to 2D cultures and may induce the expression of responsive genes, such as hypoxia-inducible factor and vascular endothelial growth factor. Findings, therefore, demonstrating cancer cells cultured in 3D better recapitulate in vivo behaviors than 2D cultures.[2,85] Synthetic biomaterial scaffolds offer better outcomes for studies that require tailoring of the tumor microenvironment (eg, mechanical properties and surface chemistry).[4]

Other Cancer Models

Despite advances in drug development, one of the main issues that still remains is the lack of correlation between preclinical findings and clinical trial results.[86] Thus, over recent years, several groups have established BC patient-derived xenograft (PDXs) models as main tools for translational research.[87–95] For these PDX models, the tumors of the patient, acquired via biopsy or surgical resection, are sliced and transplanted in immunodeficient mice and left to grow without any in vitro manipulation. Consequently, generations of mice are used for drug-testing purposes in an effort to advance patient therapy.[96] These models are also known as personalized mouse models or patient-derived tumor xenografts models to acquire further understanding on tumor progression, metastasis, and eradication via key targets. PDXs generated from BC tumor samples have been shown to mimic several tumor behaviors and characteristics of the original tumor.[87,89,95] Moreover, in the past 20 years, there have been several improvements in BC PDXs. For instance, DeRose et al[89] incorporated the addition of mesenchymal stem cells (MSCs) to modify the microenvironment, while other groups such as Kabos et al[97] incorporated Matrigel with the PDXs models. Additionally, studies by Liu et al[98] were able to show that CD44+ cancer stem cells (CSCs) directly metastasize to the lungs and lymph nodes using PDXs indicating that breast cancer stem cells (BCSCs) characterization could be performed using PDXs models.

Unfortunately, PDXs have several limitations. One issue is the rapid loss of human stroma that becomes replaced by murine stroma following engraftment,[89] potentially leading to change in the tumor biology.[99] Considering the importance of immune cells during tumorigenesis and BC metastasis, another main issue with PDXs is the need to use immunocompromised mice. Moreover, even with the immunocompromised mice, PDXs models have low engraftment rates and require a long time (several months) to be established. Additionally, in comparison to regular tissue culture techniques, PDX models are significantly more costly since they require production of genetically engineered mice and their maintenance. Thus, overall PDXs have not led to major enhancement in the survival rate of patients with cancer and needs further improvements.[100]

Applications

Many cancers have been shown to metastasize to bone, and this topic has been intensely raised in the current research leading to novel models to establish the multiple stages of metastasis.[101,102] It is now well known and accepted that in the primary stages of metastasis, disseminated tumor cells in the bone undergo a prolonged period of growth arrest in response to cues from the bone marrow (BM) microenvironment, following the successful removal of the primary tumor, this is also known as "dormancy".[28] Dormancy has been implicated with cell cycle arrest and drug resistance, and engineering of dormant stage has emerged as a novel clinical approach to tackle BC. This clinical behavior is frequently observed in cancers of the breast,[103] along with other cancers (skin[104] and prostate[105]) with relapse time varying from years to decades. In the case of BC, bone is the most common site of metastasis, affecting up to 70% of women with advanced disease.[106] The progression of this disease results in severe skeletal complications with an average 5-year survival of only 20% of the patients.[107]

Despite several improvements, the mechanisms underlying BC homing to bone remains poorly understood. Primary breast tumors can transform to invasive BC. This transformation of the cells is also known as EMT.[108,109] During EMT, the BCCs lose their epithelial phenotypes including their

polarity and specialized E-cadherin-based cell–cell contacts, and they acquire a migratory phenotype, which is associated with an increase in metastatic potential.[110,111] Recently, CSCs have been described as inducers of EMT in tumor cells.[112] Furthermore, Phillips et al[113] have demonstrated that cells undergoing EMT may be chemoresistant and have linked this resistance to the presence of CSCs within this population of transformed cells. Additionally, the EMT process allows CSCs to maintain their self-renewal abilities leading to a more invasive phenotype capable of initiating secondary tumors at distant sites.[114] Studies by Louie et al[115] have linked BC with EMT by using BCSCs conferring enhanced invasiveness, conserved EMT properties, and stem-like properties in an immune-deficient mouse model. It is generally established that dormant tumor cells can stay in a nondividing phase for many years as single cells with chemoresistance and radiation resistance characteristics.[116–119] Hence, BC resurgence is believed to be a result of BCSCs remaining dormant within the bone microenvironment. BCSCs, as described by Al-Hajj et al,[87] possess several other characteristics such as long-term self-renewal, chemoresistance, and ability to initiate distant metastatic disease.[120] There is a growing need to understand this mechanism of dormancy in order to develop therapies to target these dormant cells.[3,63,87,117,121–137] In the literature, there are two categories of dormancy. First is cellular dormancy, where single cells enter into a nonproliferative state. Cells are described to be quiescent and can show G0/G1 cell cycle arrest in response to microenvironmental signals or stresses.[102] Second is tumor mass dormancy in which the growth of the tumor mass is limited by a state of matched turnover between proliferative and apoptotic cells.[138] BC bone dormancy in vitro models have fundamental limitations with regard to reproducibility and flexibility of design. To date, many 2D in vitro models and in vivo models have been used to investigate the tumor microenvironment;[139,140] however, the complexity of human bone is difficult to recapitulate.[141,142]

Although 2D models present easy and powerful methods for investigating BCC behavior in vitro, a 2D cancer postmetastasis model oversimplifies the native 3D microenvironment due to the lack of spatial cues.[50] In addition, a 2D cell–cell in vitro model often fails to mimic cellular interaction with native ECM. This lack of cell–matrix interactions can affect gene expression of both normal and cancerous cells.[143] Some groups have demonstrated 3D models of cancer cell dormancy (Table 1). For instance, Weaver et al[144] showed that blocking β1 integrins can lead to BCCs entering a nonproliferative phase. Similarly, to represent the premetastatic niche, Ghajar et al[145] have recently fabricated microvascular constructs made of stromal cells derived from the BM and ECs in order to have a variety of cell populations present in their model to represent the metastatic niche. Their findings suggest that the presence of the ECs reduced proliferation of cancer cells by fivefold. Moreover, dormant cells versus active cells were found to have different proliferation potential with active cells being able to proliferate more. Moreover, progress has been made in fabricating bioreactors with the potential of mimicking the architecture observed in the bone.[20,146,147] In these studies, BCCs were cultured and observed to form a "single cell file" that is known to be characteristic of metastatic cancer in vivo. Findings also showed that the growth rate of BCCs was reduced in this 3D

Table 1. A summary of models studying breast cancer dormancy.

MODELS FOR BREAST CANCER DORMANCY	SIGNIFICANT FINDINGS AND LIMITATIONS	REFERENCE
Investigated dormancy of BCCs in cocultures with bone marrow cells on 3D collagen porous scaffold (Gelfoam) both *in vitro* and *in vivo*.	Identified bone marrow stromal cells in co-culture with BCCs supported proliferation whereas other bone marrow cell lines were inhibitory. Validated findings in vivo. Bone marrow cell lines were derived from other sources—HUVEC, immortalized fetal osteoblasts and stromal cells, which may not be representative of the cells in the native adult bone marrow microenvironment.	149
Fabricated 3D scaffolds consisting of micron-sized random and aligned fibers to mimic the orientation and size of collagen fibers in the native ECM.	Investigated proliferation, viability and cell cycle analysis of BCCs on electrospun fibrous scaffold and determined the aggressive BCCs adopt a dormant phenotype, while chemoresistant BCCs retained their dormant phenotype. Co-cultures with other cells types were not examined.	150
Examined the influence of β1- and β4-integrins on BCC behavior in a 3D basement membrane (Matrigel).	Demonstrated that integrins regulated the level of the acini organization and reverted the malignant phenotype to a normal phenotype. Matrigel is derived from tumor basement membrane and can vary in protein/growth factor content.	144
Investigated BCC behavior in metastasis assay in mice and in an organotypic microvascular culture.	Determined dormant BCCs reside upon microvasculature of lung, bone marrow and brain *in vivo* and endothelial cells via thrombospondin-1 induces sustained BCC quiescence. HUVEC cells used in *in vitro* model may not represent native adult endothelium.	145

environment, while osteoblasts in the coculture altered the phenotype in response to the metastatic invasion, adopting a more cuboidal morphology.[148] As a model of cancer dormancy, Marlow et al fabricated a 3D coculture model by culturing MSCs together with ECs and BCCs in a 3D collagen matrix. BCCs in cocultures proliferated less than in monocultures and appeared to be cell cycle arrested (Fig. 10).[149]

Recently, others and we have shown that 3D in vitro models using synthetic scaffolds can overcome the limitations of 2D models.[150,151] Our studies using MDA-MB-231 BCCs seeded on the 3D scaffolds showed changes in cell morphology, adherence, and growth. Unlike cells on fibrous scaffolds, cells seeded on TCP surfaces displayed confluency by day 7 (Fig. 11A). Furthermore, the aggressive MDA-MB-231

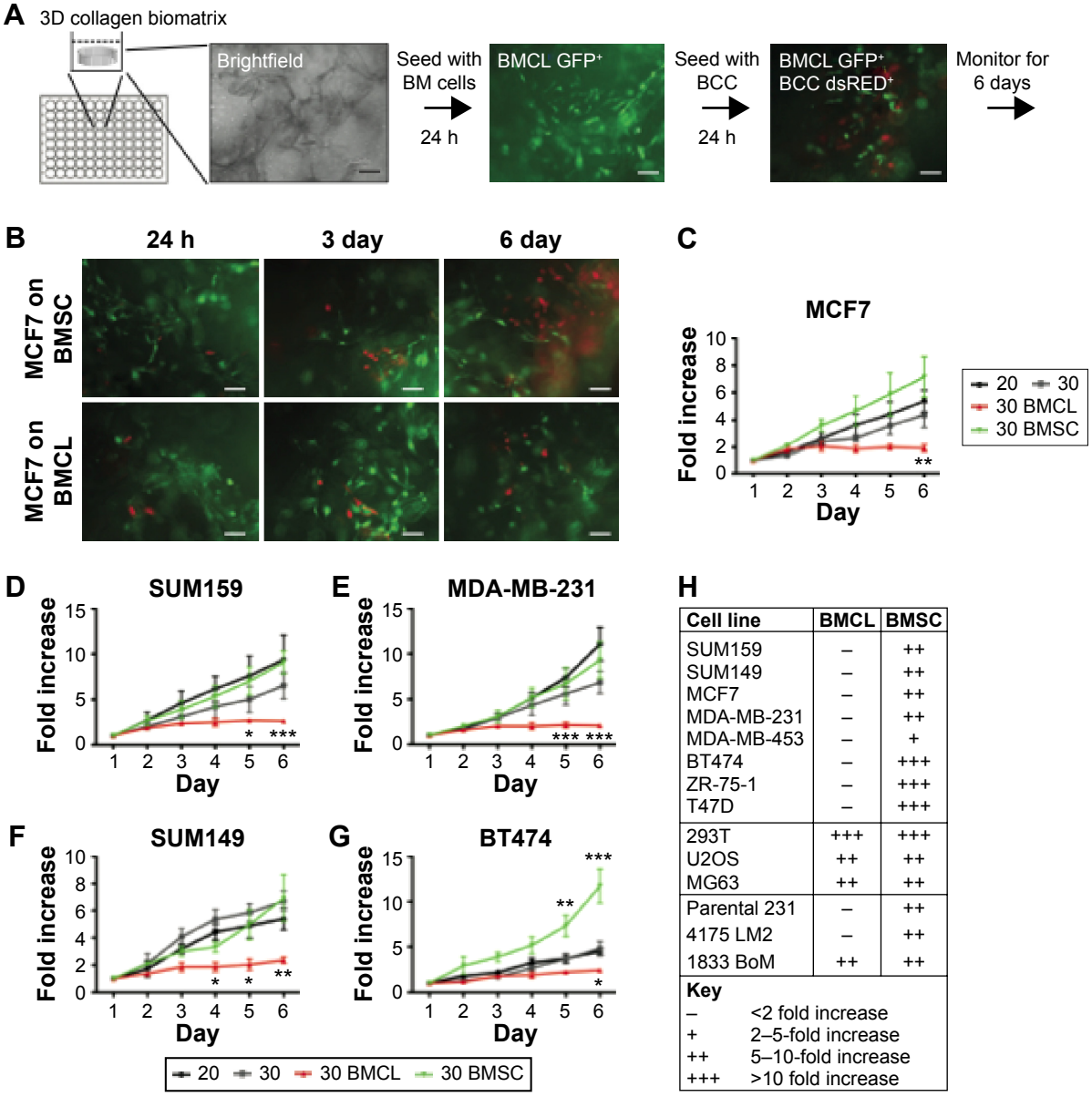

Figure 10. Bone marrow (BM) metastatic niche models: primary bone marrow mesenchymal stem cell (BMSC) supported proliferation of breast cancer cells (supportive niche), whereas a mix of osteoblasts, mesenchymal, and endothelial cell lines (BMCLs) did not support proliferation of breast cancer cells (inhibitory niche). (**A**) Diagram of bone marrow niche model setup. The 3D biomatrix is seeded with GFP+ BMCL or BMSC when bone marrow cells reach subconfluency; dsRED+ BCCs are seeded in low numbers. (**B**) Representative images of dsRED+ MCF7 cells grown into GFP+ BMCL (bottom) or BMSC (top) over six days. Scale, 50 μm. (**C**–**G**) Total fluorescence of BCCs (MCF7, SUM159, MDA-MB-231, SUM149, and BT474) was monitored. BCCs seeded in 3D biomatrix without stromal cells (3D monoculture, 3D) and BCCs plated in standard 2D conditions (2D) were used as controls. Fluorescence is expressed as the fold increase from 24 hours after seeding ($n = 6$ experiments, five replicates in each experiment). Error bars, standard error of the mean. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; two-way analysis of variance with Bonferroni posttest. (**H**) Proliferation rates of cell lines in 3D coculture. Only BCCs were found to be growth arrested in the inhibitory niche (BMCL). ER+ BCCs are indicated in bold. The fetal kidney cell line, 293t, and the osteosarcoma cell lines, U2OS and MG63, proliferate in the BMCL coculture. The subline of MDA-MB-231 1833 BoM proliferates in BMCL coculture, the parental and the 4175 LM2 lines do not.[149]

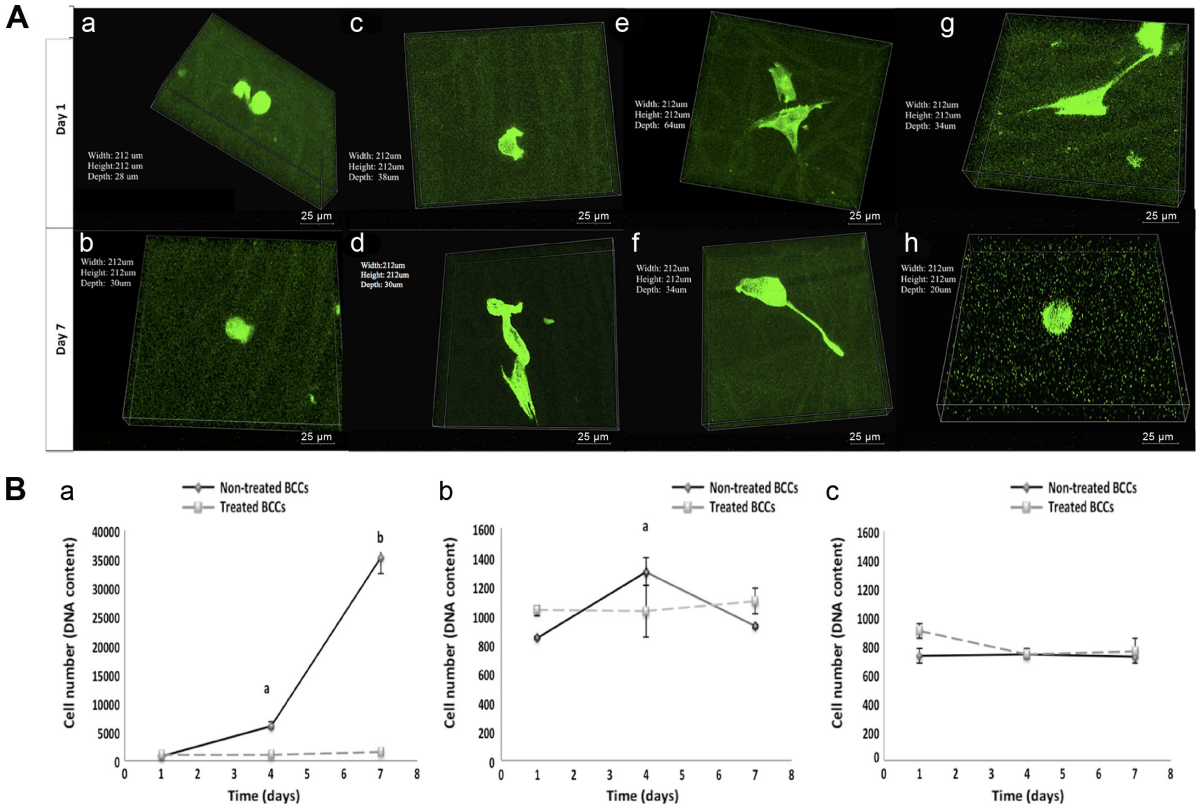

Figure 11. BCCs morphology and growth on polycaprolactone (PCL) random and aligned scaffolds. (**A**) Confocal fluorescent microscope images of MDA-MB-231 cells on the PCL random and aligned fibrous scaffolds and TCP control. Volume view of MDA-MB-231 BCCs, green indicates F-actin. On random fibers, nontreated cells at (a) day 1 and (b) day 7 and treated cells at (e) day 1 and (f) day 7. On aligned fibers, nontreated cells at (c) day 1 and (d) day 7 and treated cells at (g) day 1 and (h) day 7. 60× objective. Scale bar is 25 μm. The arrows show the cell body orientation along the fibers. (**B**) BCC growth on random and aligned fibrous scaffolds in comparison to TCP. (a) TCP. $^{a}P < 0.05$, significant increase in growth of nontreated BCCs at day 4 as compared to day 1. $^{b}P < 0.05$, significant increase in growth of nontreated BCCs at day 7 as compared to day 1 and day 4. (b) Random fibers. $^{a}P < 0.05$, significant increase in growth of nontreated BCCs at day 4 as compared to day 1 and day 7. (c) Aligned fibers. Values are mean ± standard deviation.[150]

cells showed little to no change in cell number over time on the scaffolds, whereas on TCP, they displayed a significant increase in cell number at days 4 and 7 as compared to day 1 ($P < 0.05$) (Fig. 11B), indicating that the scaffolds may support dormancy. Pathi et al[152] fabricated a 3D PLG scaffold and illustrated that hydroxyapatite nanoparticles play a crucial role in regulating BC bone metastasis. Moreover, the ability of malignant cells to grow and home to the BM in vivo depends upon specific cell–cell and cell–ECM interactions, many of which are absent when cells are cultured on conventional 2D tissue culture plastic.[153] Currently, the cell-based 2D monolayer cultures used as in vitro models present several limitations that 3D tissue engineering scaffolds/models can address.[17,34,154,155]

Conclusion

A growing need exists for the development of novel in vitro systems to facilitate the discovery of innovative therapies for BC. In order to realistically mimic the tumor microenvironment, 3D systems can be designed to allow for complex interactions between multiple cells and cell–ECM. As mentioned in this review, current animal models have several limitations that 3D tissue engineered scaffold models can potentially address. It is feasible to develop 3D culture systems that can be specifically designed to recapitulate key characteristics of the tumor tissue to model specific disease stages of BC (EMT, metastasis, invasion, dormancy) and important BC hallmarks including several layers of complexities (coculture with immune cells, bone cells, growth factors, etc). The 3D scaffolds can also be used in conjunction with high-tech microfluidic system devices to acquire highly effective models to screen and target CSCs within different milieu. These efforts would most likely be less expensive than the cost of animal studies. The 3D culture systems have been established and shown to be an improvement over 2D monolayer cultures in several aspects (ie, drug response, model of invasion and metastasis, model of EMT, and recently model of dormancy). Thus, tissue engineering can advance the development of relevant in vitro models for BC research.

Abbreviations

BC, breast cancer; BCCs, breast cancer cells; BCSCs, breast cancer stem cells; BM, bone marrow; CSCs, cancer stem cells; EC, endothelial cells; ECM, extracellular matrix; EMT,

epithelial to mesenchymal transition; HUVEC, human umbilical vein endothelial cells; MSCs, mesenchymal stem cells; PLG, poly(lactide-co-glycolide); TCP, tissue culture polystyrene; 3D, three-dimensional; 2D, two-dimensional.

Author Contributions

Wrote the first draft of the manuscript: KG and TLA. Contributed to the writing of the manuscript: KG and TLA. Agree with manuscript results and conclusions: KG and TLA. Jointly developed the structure and arguments for the paper: KG and TLA. Made critical revisions and approved final version: TLA. All authors reviewed and approved of the final manuscript.

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