Engineering 7 (2021) 1375–1380

Contents lists available at ScienceDirect

Engineering

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

Research
New Technology of Tumor Diagnosis and Treatment–Review

The Fibrillar Matrix: Novel Avenues for Breast Cancer Detection and Treatment

Rasha Rezk [a,b,#], Raquel Marín-García [a], Annica K.B. Gad [a,c,*,#]

[a] Weston Park Cancer Centre, Department of Oncology and Metabolism, The Medical School, The University of Sheffield, Sheffield S10 2RX, United Kingdom
[b] Department of Engineering, University of Cambridge, Cambridge CB2 1PZ, United Kingdom
[c] Madeira Chemistry Research Center, University of Madeira, Funchal 9020105, Portugal

ARTICLE INFO

Article history:
Received 9 November 2020
Revised 28 February 2021
Accepted 27 April 2021
Available online 4 August 2021

Keywords:
Breast cancer
Tissue stiffness
Cancer metastasis
Cell migration
Bioengineered scaffold
Viscosity

ABSTRACT

Breast cancer is marked by large increases in the protein fibers around tumor cells. These fibers increase the mechanical stiffness of the tissue, which has long been used for tumor diagnosis by manual palpation. Recent research in bioengineering has led to the development of novel biomaterials that model the mechanical and architectural properties of the tumor microenvironment and can be used to understand how these cues regulate the growth and spread of breast cancer. Herein, we provide an overview of how the mechanical properties of breast tumor tissues differ from those of normal breast tissue and non-cancerous lesions. We also describe how biomaterial models make it possible to understand how the stiffness and viscosity of the extracellular environment regulate cell migration and breast cancer metastasis. We highlight the need for biomaterial models that allow independent analysis of the individual and different mechanical properties of the tumor microenvironment and that use cells derived from different regions within tumors. These models will guide the development of novel mechano-based therapies against breast cancer metastasis.

1. Introduction

Breast cancer is the second most commonly diagnosed cancer in women worldwide. In 2018, the global number of new cases exceeded two million, and over 626 000 patients died from the disease, according to GLOBOCAN 2018[†]. The leading cause of death is metastasis of the breast tumor to distant organs and the resulting dysfunction of the organs. To metastasize, breast tumor cells must invade the surrounding tissue and migrate away from the primary tumor tissue. This motile capacity of cells is driven by forces that cells exert on the extracellular environment and by the mechanical properties of the extracellular microenvironment. The forces of cells can deform and reorganize their microenvironment, and the stiffness of the environment can regulate these cellular forces [1]. The spatial location of cells in the tumor also influences their migratory behavior [2]. Understanding the feedback mechanism(s) through which cellular forces regulate the local extracellular environment, and how the stiffness of the environment regulates cells, will lead to a greater understanding of the development, progression, and metastasis of breast cancer.

The extracellular stiffness, architecture, and organization of tissues have profound influences on tissue function in both healthy and pathological contexts [3]. For example, these properties change during the initiation and progression of cancers, as well as during cell invasion and metastasis to distant sites [4]. Weaver and colleagues, as reviewed by Kumar and Weaver [4], pioneered the field of breast cancer research by highlighting the mechanical causes of the disease. Indeed, the stiffening of breast tissue and the progressive loss of tensional homeostasis can be used to detect tumors [5]. In addition, approaches based on the physical properties of cancer cells can contribute to new treatment strategies and to the development of new diagnostic tools and cancer treatments [6].

Several lines of research have focused on understanding the role of extracellular mechanics in breast cancer invasion, dissemination, and response to treatment, often using approaches based on bioengineered three-dimensional (3D) materials. In this brief review, we describe the mechanical characteristics of breast cancer tissues and recent advances in 3D biomaterials that can mimic the

* Corresponding author.
E-mail address: a.k.gad@sheffield.ac.uk (A.K.B. Gad).
[#] These authors contributed equally to this study.
[†] https://www.uicc.org/news/global-cancer-data-globocan-2018

https://doi.org/10.1016/j.eng.2021.04.024

responses of cells to the stiffness, density, and viscosity of the microenvironment. We further highlight the importance of taking the heterogeneity within and between tumors and patients into account in order to model the interactions between tumor cells and the microenvironment, and to guide the development of novel mechano-based therapies against cancers.

2. Stiffness as a biomarker for breast cancer

Malignant breast tissue is stiffer than normal tissue [7]. This substantial increase in the stiffness of the breast tissue has long been used for palpation-based diagnosis. Breast cancer is a very heterogeneous and complex disease with a wide range of morphological features, immunohistochemical profiles, and unique histopathological subtypes, all of which have specific clinical courses and outcomes [8]. These diverse subtypes can therefore have different and wide-ranging mechanical properties. Invasive ductal carcinoma is the most common form of invasive breast cancer, accounting for more than 50% of breast cancers [8,9]; invasive carcinoma and carcinoma *in situ* are classified as ductal or lobular, based on the site from which these tumors originate [8].

To provide an overview of the literature showing how the stiffness of breast cancer tissues differs from that of the tissues of the normal breast and non-cancerous lesions, we performed a systematic literature search. Table 1 [7,10–15] provides representative examples from the identified literature showing the ranges of stiffness for different histological types of breast cancer, normal breast tissue, and non-malignant breast lesions. Methods used to analyze tissue stiffness include atomic force microscopy (AFM) and shear wave elastography (SWE), which can be used to differentiate between invasive and noninvasive tumor tissue. Although the stiffness range varies according to the method used, it is clear that breast cancer tissues show increased stiffness when compared with various types of normal tissue and non-cancerous lesions, both *in vivo* and *ex vivo*. In addition, the data indicate increased stiffness in invasive cancer compared with noninvasive cancer (Table 1).

The stiffness of body tissues is mainly governed by the stiffness of the extracellular stroma, which is a fibrillar matrix of collagen and other extracellular matrix proteins and molecules. The production and/or crosslinking of these components increases the stiffness and density of the tissue [16,17], which correlates with progression in breast cancer [16,18]. Provenzano et al. [19] showed that the increased density of collagen promotes the initiation and progression of mammary tumors *in vivo*. They also reported a strong correlation between collagen fibers that were oriented radially from—instead of aligned with—the tumor boundary, and local invasion of the tumor [19]. This increased density and alignment of collagen fibers has been further linked to poor prognosis and can be used as a prognostic marker for these patients [20]. However, whether the density of collagen contributes to prognosis remains unclear. Indeed, a large study of 9232 women diagnosed with primary invasive breast carcinoma from 1996 to 2005 failed to identify any correlation between mammographic breast density and risk of death from breast cancer [21].

Table 1
Studies determining the main stiffness characteristics of normal breast tissue, benign lesions, and tumor tissue in the adult human breast, as analyzed by atomic force microscopy (AFM), shear wave elastography (SWE), and B-mode ultrasound (B-US).

Reference	Method	*In vivo*/*ex vivo*	Tissue types	Mean stiffness (kPa)	Stiffness range (kPa)
Acerbi et al., 2015 [7]	AFM	*Ex vivo*	Normal breast tissue	0.4	Up to 2–3; invasive
			Invasive ductal carcinoma	> 5	Core 1–2; invasive rim up to 10
Ansardamavandi et al., 2016 [12]	AFM	*Ex vivo*	Normal breast tissue		
			Cellular region	0.7	
			Fibrous/extracellular	16.05	
			Intermediate region containing ducts, fibers, lumens, fatty tissues	5.19	
			Grades 2 and 3 breast carcinomas		
			Cellular region	1.42	
			Fibrous/extracellular	14.45	
			Intermediate region containing ducts, fibers, lumens, fatty tissues	5.48	
Berg et al., 2015 [13]	SWE	*In vivo*	Fibroadenomas	45	30–79
			Ductal carcinoma *in situ*	126	71–180
			Invasive lobular carcinomas	180	124–180
			Invasive ductal carcinoma	180	158–180
Chang et al., 2011 [11]	SWE	*In vivo*	Fibroadenomas	49.58 ± 43.51	5.89–192.51
			Ductal carcinoma *in situ*	117.75 ± 54.72	46.95–193.30
			Invasive lobular carcinoma	169.50 ± 61.06	107.63–283.84
			Invasive ductal carcinoma	157.50 ± 57.07	58.34–300.00
Suvannarerg et al., 2019 [14]	SWE	*In vivo*	Benign lesions	19.73	5.15–104.10
			Ductal carcinoma *in situ*	37.85	4.25–255.50
			Invasive lobular carcinoma	105.75	24.05–171.65
			Invasive ductal carcinoma	96.65	8.20–281.95
Rabin and Benech, 2019 [15]	B-US	*In vivo*	Normal breast tissue	24.7 ± 8.1	
			Invasive ductal carcinoma	98.1 ± 12.9	
Tian et al., 2017 [10]	SWE	*In vivo*	Benign lesions of fibroadenomas, fibroadenomatous hyperplasias, cystic hyperplasias, papillomas, adenosis, mammary duct ectasia, chronic inflammations, fat necrosis	78	48.0–110.7
			Invasive ductal carcinomas, ductal carcinomas *in situ*, mucinous carcinomas, invasive lobular carcinoma, intraductal papillary carcinomas	185.40	154.9–220.0

In the studies described above, it was not possible to determine whether it is the amount and density of collagen or the tissue stiffness *per se* that regulates tumor invasion. To determine the relationship between tissue stiffness and tumor progression, Fenner et al. [22] resected tumors from mouse models of breast cancer and analyzed the bulk moduli of the freshly excised intact tumors *ex vivo*. Unlike previous studies, they reported a clear inverse correlation between the bulk modulus of the resected tumors and subsequent local recurrence and metastasis; furthermore, they reported that tumor stiffness correlated with the amount of collagen in the tissue. It is also important to note that Levental et al. [18] used a mouse model of breast cancer to show that increased collagen crosslinking was related to increased invasion of tumor cells, with no change in collagen levels. Taken together, these findings emphasize that the effects of the extracellular matrix on cell behavior are complex; they also highlight that collagen density, amount, alignment, crosslinking, and spatial organization in relation to the surface of the epithelial component of the tumor should be considered to determine whether compliance can be used as a biomarker for breast cancer.

3. Extracellular matrix stiffness and breast cancer

To understand how the mechanical properties of the extracellular matrix regulate oncogenic cell transformation and tumor cell invasion, these properties need to be examined without changing the chemical composition or architectural properties. To this end, synthetic fibrous materials, polyacrylamide hydrogels, and polydimethylsiloxane elastomer-coated substrates have been developed. Chaudhuri et al. [23] showed that normal, MCF10A mammary epithelial cells that are exposed to increased matrix stiffness have a phenotype similar to that of oncogenically transformed cells. This finding is in line with the observation that the stiffness of the matrix in a 3D culture model of breast cancer affects the accessibility of the genome and induces malignancy [24].

Endothelial cell sprouting and blood vessel formation is required for the growth and metastasis of breast cancers. In contrast to epithelial cells, endothelial cells showed decreased proliferation, invasion, and sprouting when exposed to a gelatin-methacrylate and collagen matrix with increased stiffness and constant collagen density [25]. In the same work, Berger et al. [25] reported that increased stiffness resulted in a gradual decrease in cell sprouting at 1.5 and 3.0 $mg{\cdot}mL^{-1}$ of collagen I. In the absence of collagen, stiffness promoted cell sprouting, with a peak at 7 kPa; however, cell sprouting was reduced above this stiffness, reaching near zero at 12 kPa.

Collagen type I can influence cell responses to tissue stiffness, and these findings by Chaudhuri et al. [23] and Berger et al. [25] highlight the importance of taking the chemical composition of the material into account at any given stiffness.

In line with these observations, it should be noted that the responses of cells to mechanical cues depend on the type of cell under study [10,26]. The stiffness of extracellular fibers regulates the migration speed of a large variety of cells, such as metastatic MDA-MB-231 breast cancer cells, UM-SCC-74B squamous cell carcinoma cells, HT1080 fibrosarcoma cells, and NIH3T3 fibroblasts. However, the optimal fiber stiffness for the speed of cell migration varies considerably between different cell types [27]. Computational modeling based on experimental data has suggested that cells exhibit their maximal speed of migration at an intermediate range of fiber stiffness, and that the optimal stiffness for maximal speed varies between cell types [26]. This concept is supported by Wang et al. [27], who demonstrated an optimal stiffness of synthetic 3D fibers for the maximum speed of cell migration. It is worth noting that the type of migration observed on these fibers was a "slingshot" movement rather than the traditional mesenchymal migration. In addition, recent observations highlight the possibility that it is not the increased stiffness of the extracellular environment or of fibers in three dimensions, but rather the increased fiber density that promotes the changes of stromal cells that often are observed in cancer [28,29]. Taken together, these observations emphasize the importance determining the optimal stiffness for a given matrix composition and dimensionality for a specific cell type. Identification of the optimal stiffness for cell migration *in vivo* and the underlying molecular mechanisms in its regulation would make it possible to identify relevant biomarkers for invasive tumor regions, which could guide the development of novel mechano-based anti-cancer therapies.

4. The impact of the viscous properties of breast cancer

As described above, recent research in bioengineering has led to the development of novel biomaterials that can be used to mimic the elastic modulus and architecture of the tumor microenvironment. However, the tissues in our bodies do not behave like an elastic solid; instead, they have both viscous and elastic characteristics. It is therefore important to consider other mechanical properties, such as viscosity.

Breast cancer tissues have been found to be more viscous, or fluid-like, than tissues from benign lesions [30], which aligns with the observation that the production of hyaluronan, a molecule that governs the water content of tissues, is upregulated in and linked to poor prognosis of breast cancer [31]. Magnetic resonance elastography has demonstrated significant differences in viscoelasticity between malignant and benign breast tumors [30], as well as between glioblastoma and healthy brain parenchyma [32]. MCF-7 breast cancer cells show reduced viscosity and elasticity, which indicates that breast cancer cells are more fluid and "softer" than their benign counterparts [33]. A further study using MCF-10A, MCF-7, and MDA-MB-231 cells showed that normal breast epithelial cells have greater viscosity than tumor cells; the actin distribution and the greater nucleus-to-cytoplasm ratio of the tumor cells are the two main factors in the determination of cell viscosity [34]. Tumor cell metastasis depends on various factors, which include remodeling of the extracellular matrix and the potential for deformation of the nucleus [35,36]. However, it should be noted that on very rigid substrates, viscosity has little effect on cell attachment and spreading [37]. Viscosity playing a role in the spread of cancer is supported by the observation that while cancer cells are unable to squeeze and migrate through a very rigid pore size environment [35,38], they can migrate through nanoporous matrices that exhibit sufficient mechanical plasticity [39].

Recently developed hydrogels offer the possibility of tuning the stress relaxation or loss modulus independently from the elastic modulus [40–44]. Several cell types respond to viscoelastic substrates as though they were softer than purely elastic substrates of the same elastic modulus [44]. For example, fibroblasts and cancer cells were unable to spread on soft elastic gels; however, they were able to spread on soft viscoelastic gels through the β1 integrin receptor protein, myosin, and Rho, exhibiting robust focal adhesions and stress fibers and enhanced activation of the transcriptional regulator protein YAP, similar to their behavior on stiff and elastic substrates [45]. Increased stress relaxation promotes cell spreading, proliferation, and the osteogenic differentiation of mesenchymal stem cells in 3D culture [42]. These observations align with the observation that viscosity can have a profound effect on cell morphology, adhesion, proliferation, and differentiation [43]. Taken together, these observations indicate that it is important to include both the viscous and elastic properties when developing

an understanding of the regulation of tumor invasion and metastasis.

Engineered biomaterial *in vitro* models with independently tunable viscoelastic properties provide new avenues toward an understanding of the time-dependent aspects of extracellular matrix mechanics in the regulation of cell behavior and metastasis. In addition, these models provide an efficient reductionist approach for studying the impact of different extracellular mechanical cues and a wide range of extracellular proteins on tumor development and progression. For example, chemically defined hydrogels and synthetic fibers can be functionalized with collagen or fibronectin [44], or with arginine–glycine–aspartic acid (RGD) peptides [28,45], to study the impact of elasticity and viscosity on cell adhesion and motility. Table 2 [23,28,46–49] lists examples of preclinical biomaterial *in vitro* models that can be used to study the impact of extracellular mechanical cues on breast cancer.

5. The impact of intra-tumoral mechanical heterogeneity

It is important to note that the same biomaterial model will result in different effects on different cell types [27]; furthermore, the same cell type derived from different patients will respond differently to the same stiffness [50,51]. Therefore, understanding inter- and intra-tumoral heterogeneity at the cellular level can be key in understanding cancer development, progression, and treatment failure.

Most of the aforementioned 3D models have used immortalized cell lines, many of which have been cultured on glass or plastic for decades. These cell lines are therefore likely to have developed properties that facilitate their growth under these non-physiological—and very stiff—culture conditions. Thus, these cells should not be considered as true representatives of tumor cells in patients, and it is likely that their responses to mechanical cues will differ from those of tumor cells *in vivo*. It has been observed that glioblastoma cells derived from different regions within a tumor show different mechanical behaviors in standardized controlled settings, which further adds to the complexity of the mechanical heterogeneity of tumor cells and how this heterogeneity can regulate tumor progression and infiltration [50].

Several observations have suggested that the material properties of breast tumors depend on the region of location within the tumor. For example, tumors have a core that is almost as compliant as normal tissue, whereas the invasive tissue at the rim is stiffer [7,52]. The causes of this intra-tumoral heterogeneity remain unclear. It is therefore important to sample and test cells from different tumor regions under standardized conditions. Doing so will assist researchers in understanding how much of the phenotypic variability between cells in different parts of a tumor is due to the cell types themselves, and how much is due to the environment [50].

Although the tumor stroma is the main determinant of the stiffness of tumor tissue, it should be noted that the tumor cells, immune cells, cancer-associated fibroblasts, endothelial cells, and necrotic areas also contribute to the mechanical properties of the tissue. It has been observed that most tumor cells with low migration and invasion potential show a five-fold greater stiffness than migratory and invasive tumor cells [53], which suggests that reduced cell stiffness promotes metastasis. In a ground-breaking finding, Kenny and Bissell [54] reported that a normal extracellular environment can revert the phenotype of an oncogenically transformed cell back to normal, which suggests that the phenotype of the extracellular environment can override the malignant genotype of a cell. It is therefore important to further clarify how much of the behavior of cells is due to gene expression, and how much is regulated by the surrounding environment.

Taken together, the studies described above highlight the need to analyze cells in different stages of oncogenic transformation and from different tumor regions under standardized extracellular conditions, using, for example, fibers with defined mechanical, chemical, and spatial properties. In addition to this intra-tumoral heterogeneity, it should be considered that breast cancer is a heterogeneous disease, and that different breast cancer types have different and unique features.

6. Identification of biomarkers for molecular diagnosis, patient prognosis, and targeted therapies

Materials that can mimic the mechanical properties of the extracellular environment would permit the identification of the mechanical conditions required for tumor cell invasion and the underlying molecular mechanisms of this regulation. These mechanisms could then be used to develop novel biomarkers and molecular targets that could be used to control tumor invasion via regulation of the cell mechanics. Berger et al. [55] used a matrix composed of methacrylate gelatin and collagen I to show that cell invasion into stiff matrices depends upon the extracellular protein fibronectin. They further showed that, compared with normal tissue, a domain of fibronectin that is overexpressed in invasive breast cancer cells can promote cell invasion *ex vivo* [55]. Targeting this domain of fibronectin therefore represents a therapeutic strategy. This finding can also be used to develop diagnostic strategies to identify patients who have more invasive tumors and therefore require more aggressive treatments.

As mentioned above, the phenotype of oncogenically transformed malignant cells can be reverted to a more normal phenotype through normalization of their microenvironment [54].

Table 2
Examples of preclinical two-dimensional (2D) and 3D *in vitro* biomaterial cancer models, including the model, material, and cell type.

Reference	2D or 3D model	Materials	Cells
Chaudhuri et al., 2014 [23]	Interpenetrating networks of alginate and reconstituted basement membrane matrix	Matrigel, alginate	MCF-10A cells
Chopra et al., 2014 [46]	Polyacrylamide gels	Polyacrylamide, fibronectin	Neonatal ventricular rat myocytes, human mesenchymal stem cells, 3T3 fibroblasts,
	Hyaluronan gels	Hyaluronan, fibronectin	human umbilical vein endothelial cells (HUVECs)
Baker et al., 2015 [28]	Synthetic fibrillar extracellular matrix	Methacrylated dextran	NIH 3T3 fibroblasts and human mesenchymal stem cells
Ranga et al., 2016 [47]	Hyaluronan gels	Polyethylene glycol, hyaluronan	MCF-7 mammary carcinoma cells and C2C12 mouse myoblast cells
Kleine-Brüggeney et al., 2019 [48]	3D hydrogel beads (microfluidic droplets used to compartmentalize single cells within a hydrogel matrix)	Agarose	Pluripotent mouse embryonic stem (mES) cells
Pavel et al., 2018 [49]	3D extracellular matrix	Rat collagen I, matrigel	MCF-10A cells

Therefore, newly developed models can ideally be used to identify the stiffness and viscous conditions [23] and the physiological extracellular ligands that can revert the malignant phenotype of a cell to a more normal phenotype. Although these models offer an excellent opportunity to decouple the mechanical properties from the chemical characteristics of the extracellular matrix, they do not decisively describe whether and how stiffness regulates the progression of breast cancer. We speculate that the conflicting data described herein are due to differences between the cell types used and the chemical compositions of the materials used for the model systems. As described earlier, a problem with the current models of breast cancer is that they mainly depend on the use of immortalized tumor cell lines that do not necessarily represent the tumor cells derived from patients, where tumor cells *in vivo* are likely to show different mechanical responses. In addition to the standard classification of breast cancer cells from a patient using molecular and clinical markers, classification based on the mechanical properties of breast cancer cells can be used to provide a more personalized and specific diagnosis. Further studies will also allow researchers to understand why treatments aimed at blocking integrin receptors do not effectively block cell invasion, as well as providing novel molecular targets for treatments. The current challenges in the field of bioengineering are to create environments that mimic breast cancer tissue such that the stiffness, viscosity, and architectural cues of the cells can be tuned independently of each other, and that can be used with a wide range of cells from patients.

Acknowledgments

The authors would like to thank the Weston Park Cancer Centre (University of Sheffield, UK) the Fundação para a Ciência e a Tecnologia (FCT), the Portuguese Government (PEst-OE/QUI/UI0674/2013) and the Agência Regional para o Desenvolvimento da Investigaçaõ Tecnologia e Inovação (ARDITI), M1420-01-0145-FEDER-000005 Centro de Química da Madeira (CQM) (Madeira 14–20).

Compliance with ethics guidelines

Rasha Rezk, Raquel Marín-García, and Annica K.B. Gad declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310(5751):1139–43.
[2] Heinrich MA, Alert R, LaChance JM, Zajdel TJ, Košmrlj A, Cohen DJ. Size-dependent patterns of cell proliferation and migration in freely-expanding epithelia. eLife 2020;9:e58945.
[3] Northcott JM, Dean IS, Mouw JK, Weaver VM. Feeling stress: the mechanics of cancer progression and aggression. Front Cell Dev Biol 2018;6:17.
[4] Kumar S, Weaver VM. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. Cancer Metastasis Rev 2009;28(1–2):113–27.
[5] Butcher DT, Alliston T, Weaver VM. A tense situation: forcing tumour progression. Nat Rev Cancer 2009;9(2):108–22.
[6] Michor F, Liphardt J, Ferrari M, Widom J. What does physics have to do with cancer? Nat Rev Cancer 2011;11(9):657–70.
[7] Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, et al. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integr Biol 2015;7(10):1120–34.
[8] Makki J. Diversity of breast carcinoma: histological subtypes and clinical relevance. Clin Med Insights Pathol 2015;8:23–31.
[9] Weigelt B, Reis-Filho JS. Histological and molecular types of breast cancer: is there a unifying taxonomy? Nat Rev Clin Oncol 2009;6(12):718–30.
[10] Tian J, Liu Q, Wang X, Xing P, Yang Z, Wu C. Application of 3D and 2D quantitative shear wave elastography (SWE) to differentiate between benign and malignant breast masses. Sci Rep 2017;7(1):41216.
[11] Chang JM, Moon WK, Cho N, Yi A, Koo HR, Han W, et al. Clinical application of shear wave elastography (SWE) in the diagnosis of benign and malignant breast diseases. Breast Cancer Res Treat 2011;129(1):89–97.
[12] Ansardamavandi A, Tafazzoli-Shadpour M, Omidvar R, Jahanzad I. Quantification of effects of cancer on elastic properties of breast tissue by atomic force microscopy. J Mech Behav Biomed Mater 2016;60:234–42.
[13] Berg WA, Mendelson EB, Cosgrove DO, Doré CJ, Gay J, Henry JP, et al. Quantitative maximum shear-wave stiffness of breast masses as a predictor of histopathologic severity. AJR Am J Roentgenol 2015;205(2):448–55.
[14] Suvannarerg V, Chitchumnong P, Apiwat W, Lertdamrongdej L, Tretipwanit N, Pisarnturakit P, et al. Diagnostic performance of qualitative and quantitative shear wave elastography in differentiating malignant from benign breast masses, and association with the histological prognostic factors. Quant Imaging Med Surg 2019;9(3):386–98.
[15] Rabin C, Benech N. Quantitative breast elastography from B-mode images. Med Phys 2019;46(7):3001–12.
[16] Barcus CE, Keely PJ, Eliceiri KW, Schuler LA. Stiff collagen matrices increase tumorigenic prolactin signaling in breast cancer cells. J Biol Chem 2013;288(18):12722–32.
[17] Perepelyuk M, Terajima M, Wang AY, Georges PC, Janmey PA, Yamauchi M, et al. Hepatic stellate cells and portal fibroblasts are the major cellular sources of collagens and lysyl oxidases in normal liver and early after injury. Am J Physiol Gastrointest Liver Physiol 2013;304(6):G605–14.
[18] Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 2009;139(5):891–906.
[19] Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, et al. Collagen density promotes mammary tumor initiation and progression. BMC Med 2008;6(1):11.
[20] Conklin MW, Eickhoff JC, Riching KM, Pehlke CA, Eliceiri KW, Provenzano PP, et al. Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am J Pathol 2011;178(3):1221–32.
[21] Gierach GL, Ichikawa L, Kerlikowske K, Brinton LA, Farhat GN, Vacek PM, et al. Relationship between mammographic density and breast cancer death in the Breast Cancer Surveillance Consortium. J Natl Cancer Inst 2012;104(16):1218–27.
[22] Fenner J, Stacer AC, Winterroth F, Johnson TD, Luker KE, Luker GD. Macroscopic stiffness of breast tumors predicts metastasis. Sci Rep 2015;4(1):5512.
[23] Chaudhuri O, Koshy ST, Branco da Cunha C, Shin JW, Verbeke CS, Allison KH, et al. Extracellular matrix stiffness and composition jointly regulate the induction of malignant phenotypes in mammary epithelium. Nat Mater 2014;13(10):970–8.
[24] Stowers RS, Shcherbina A, Israeli J, Gruber JJ, Chang J, Nam S, et al. Matrix stiffness induces a tumorigenic phenotype in mammary epithelium through changes in chromatin accessibility. Nat Biomed Eng 2019;3(12):1009–19.
[25] Berger AJ, Linsmeier KM, Kreeger PK, Masters KS. Decoupling the effects of stiffness and fiber density on cellular behaviors via an interpenetrating network of gelatin-methacrylate and collagen. Biomaterials 2017;141:125–35.
[26] Bangasser B, Rosenfeld S, Odde D. Determinants of maximal force transmission in a motor-clutch model of cell traction in a compliant microenvironment. Biophys J 2013;105(3):581–92.
[27] Wang WY, Davidson CD, Lin D, Baker BM. Actomyosin contractility-dependent matrix stretch and recoil induces rapid cell migration. Nat Commun 2019;10(1):1186.
[28] Baker BM, Trappmann B, Wang WY, Sakar MS, Kim IL, Shenoy VB, et al. Cell-mediated fibre recruitment drives extracellular matrix mechanosensing in engineered fibrillar microenvironments. Nat Mater 2015;14(12):1262–8.
[29] Matera DL, DiLillo KM, Smith MR, Davidson CD, Parikh R, Said M, et al. Microengineered 3D pulmonary interstitial mimetics highlight a critical role for matrix degradation in myofibroblast differentiation. Sci Adv 2020;6(37):eabb5069.
[30] Sinkus R, Siegmann K, Xydeas T, Tanter M, Claussen C, Fink M. MR elastography of breast lesions: understanding the solid/liquid duality can improve the specificity of contrast-enhanced MR mammography. Magn Reson Med 2007;58(6):1135–44.
[31] Wu W, Chen L, Wang Y, Jin J, Xie X, Zhang J. Hyaluronic acid predicts poor prognosis in breast cancer patients. Medicine 2020;99(22):e20438.
[32] Streitberger KJ, Sack I, Krefting D, Pfüller C, Braun J, Paul F, et al. Brain viscoelasticity alteration in chronic-progressive multiple sclerosis. PLoS ONE 2012;7(1):e29888.
[33] Wang Y, Xu C, Jiang N, Zheng L, Zeng J, Qiu C, et al. Quantitative analysis of the cell-surface roughness and viscoelasticity for breast cancer cells discrimination using atomic force microscopy. Scanning 2016;38(6):558–63.
[34] Nematbakhsh Y, Pang KT, Lim CT. Correlating the viscoelasticity of breast cancer cells with their malignancy. Converg Sci Phys Oncol 2017;3(3):034003.
[35] Harada T, Swift J, Irianto J, Shin JW, Spinler KR, Athirasala A, et al. Nuclear lamin stiffness is a barrier to 3D migration, but softness can limit survival. J Cell Biol 2014;204(5):669–82.
[36] Swift J, Discher DE. The nuclear lamina is mechano-responsive to ECM elasticity in mature tissue. J Cell Sci 2014;127(14):3005–15.
[37] Gong Z, Szczesny SE, Caliari SR, Charrier EE, Chaudhuri O, Cao X, et al. Matching material and cellular timescales maximizes cell spreading on viscoelastic substrates. Proc Natl Acad Sci USA 2018;115(12):E2686–95.
[38] Wolf K, Lindert MT, Krause M, Alexander S, Riet JT, Willis AL, et al. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. J Cell Biol 2013;201(7):1069–84.
[39] Wisdom KM, Adebowale K, Chang J, Lee JY, Nam S, Desai R, et al. Matrix mechanical plasticity regulates cancer cell migration through confining microenvironments. Nat Commun 2018;9(1):4144.

[40] Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, Shenoy VB. Effects of extracellular matrix viscoelasticity on cellular behaviour. Nature 2020;584 (7822):535–46.
[41] Chaudhuri O. Viscoelastic hydrogels for 3D cell culture. Biomater Sci 2017;5 (8):1480–90.
[42] Chaudhuri O, Gu L, Klumpers D, Darnell M, Bencherif SA, Weaver JC, et al. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. Nat Mater 2016;15(3):326–34.
[43] Cameron AR, Frith JE, Cooper-White JJ. The influence of substrate creep on mesenchymal stem cell behaviour and phenotype. Biomaterials 2011;32 (26):5979–93.
[44] Charrier EE, Pogoda K, Wells RG, Janmey PA. Control of cell morphology and differentiation by substrates with independently tunable elasticity and viscous dissipation. Nat Commun 2018;9(1):449.
[45] Chaudhuri O, Gu L, Darnell M, Klumpers D, Bencherif SA, Weaver JC, et al. Substrate stress relaxation regulates cell spreading. Nat Commun 2015;6: 6365.
[46] Chopra A, Murray ME, Byfield FJ, Mendez MG, Halleluyan R, Restle DJ, et al. Augmentation of integrin-mediated mechanotransduction by hyaluronic acid. Biomaterials 2014;35(1):71–82.
[47] Ranga A, Lutolf MP, Hilborn J, Ossipov DA. Hyaluronic acid hydrogels formed *in situ* by transglutaminase-catalyzed reaction. Biomacromolecules 2016;17 (5):1553–60.
[48] Kleine-Brüggeney H, van Vliet LD, Mulas C, Gielen F, Agley CC, Silva JCR, et al. Long-term perfusion culture of monoclonal embryonic stem cells in 3D hydrogel beads for continuous optical analysis of differentiation. Small 2019;15(5):1804576.
[49] Pavel M, Renna M, Park SJ, Menzies FM, Ricketts T, Füllgrabe J, et al. Contact inhibition controls cell survival and proliferation via YAP/TAZ-autophagy axis. Nat Commun 2018;9(1):2961.
[50] Rezk R, Jia BZ, Wendler A, Dimov I, Watts C, Markaki AE, et al. Spatial heterogeneity of cell-matrix adhesive forces predicts human glioblastoma migration. Neurooncol Adv 2020;2(1):vdaa081.
[51] Grundy TJ, De Leon E, Griffin KR, Stringer BW, Day BW, Fabry B, et al. Differential response of patient-derived primary glioblastoma cells to environmental stiffness. Sci Rep 2016;6(1):23353.
[52] Plodinec M, Loparic M, Monnier CA, Obermann EC, Zanetti-Dallenbach R, Oertle P, et al. The nanomechanical signature of breast cancer. Nat Nanotechnol 2012;7(11):757–65.
[53] Swaminathan V, Mythreye K, O'Brien ET, Berchuck A, Blobe GC, Superfine R. Mechanical stiffness grades metastatic potential in patient tumor cells and in cancer cell lines. Cancer Res 2011;71(15):5075–80.
[54] Kenny PA, Bissell MJ. Tumor reversion: correction of malignant behavior by microenvironmental cues. Int J Cancer 2003;107(5):688–95.
[55] Berger AJ, Renner CM, Hale I, Yang X, Ponik SM, Weisman PS, et al. Scaffold stiffness influences breast cancer cell invasion via EGFR-linked Mena upregulation and matrix remodeling. Matrix Biol 2020;85–86:80–93.