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Biomaterials to model and measure epithelial cancers

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Abstract | The use of biomaterials has substantially contributed to both our understanding of tumorigenesis and our ability to identify and capture tumour cells in vitro and in vivo. Natural and synthetic biomaterials can be applied as models to recapitulate key features of the tumour microenvironment in vitro, including architectural, mechanical and biological functions. Engineered biomaterials can further mimic the spatial and temporal properties of the surrounding tumour niche to investigate the specific effects of the environment on disease progression, offering an alternative to animal models for the testing of cancer cell behaviour. Biomaterials can also be used to capture and detect cancer cells in vitro and in vivo to monitor tumour progression. In this Review, we discuss the natural and synthetic biomaterials that can be used to recreate specific features of tumour microenvironments. We examine how biomaterials can be applied to capture circulating tumour cells in blood samples for the early detection of metastasis. We highlight biomaterial-based strategies to investigate local regions adjacent to the tumour and survey potential applications of biomaterial-based devices for diagnosis and prognosis, such as the detection of cellular deformability and the non-invasive surveillance of tumour-adjacent stroma.

Tumours are complex and heterogeneous structures. Understanding tumour progression and cancer metastasis requires the investigation of not only the tumour itself but also of the dynamic and reciprocal interactions between cancer cells and the adjacent tumour stroma, that is, the tumour microenvironment (or niche). This microenvironment is very heterogeneous but generally contains certain cell types (for example, cancer-associated fibroblasts (CAFs)), extracellular matrix (ECM) proteins and signalling molecules, which change as tumours grow and metastasize throughout the body (BOX 1). The tumour microenvironment properties are modulated, in part, as a result of alterations to the 3D fibrillar ECM that surrounds tumour tissue and to the 2D basement membrane that underlies epithelia. For example, the ECM can be modified by CAFs[1,2] and tumour cells alike, causing the matrix to become stiffer[3], more dense[4], crosslinked[5], aligned[3] and less porous[5]. In the case of larger breast tumours, patients can actually feel the stiffened tumour stroma.

Animal models are powerful systems to study the dynamic stromal properties of tumours, but it is difficult to dissect the specific contributions of individual microenvironmental cues to tumour development and progression[6]. However, reducing the in vivo niche to its major biochemical and biophysical components offers a possibility to model the tumour microenvironment in vitro. Identifying and recreating specific aspects of the tumour stroma, for example, stiffness, topography or nutrient exchange, using biomaterials allows for the fabrication of reductionist in vitro systems to study basic mechanisms that regulate cancer cell plasticity, dissemination and repopulation of the niche (BOX 2).

Biomaterials have been used to study tumour biology since the early 1980s, when scientists questioned whether signals from the extracellular compartment could regulate cell behaviour in a distinct and/or similar way as to how genetics can dictate cell fate. In particular, seminal work demonstrating that changes to the extracellular milieu could affect gene expression in mammary glands[7] has triggered unprecedented interest in how the ECM regulates cell behaviour in development. Pioneering work by the group of Mina Bissell established a 'dynamic reciprocity' between the cell and its microenvironment, showing that components of the ECM, such as collagen or fibronectin, associate with the plasma membrane and connect to the intracellular cytoskeleton through specific structures (later identified as focal adhesions). Signals from the ECM are then relayed to the nucleus to affect gene expression and to regulate the expression of ECM molecules or their modification through the expression of ECM-modifying enzymes. However, the detailed mechanisms of cell–ECM interactions are

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Box 1 | **Cancer and metastasis**

Squamous and ductal carcinoma share basic stages of cancer metastasis. These cancers originate from epithelial cells, which line surfaces and vessels of the body.

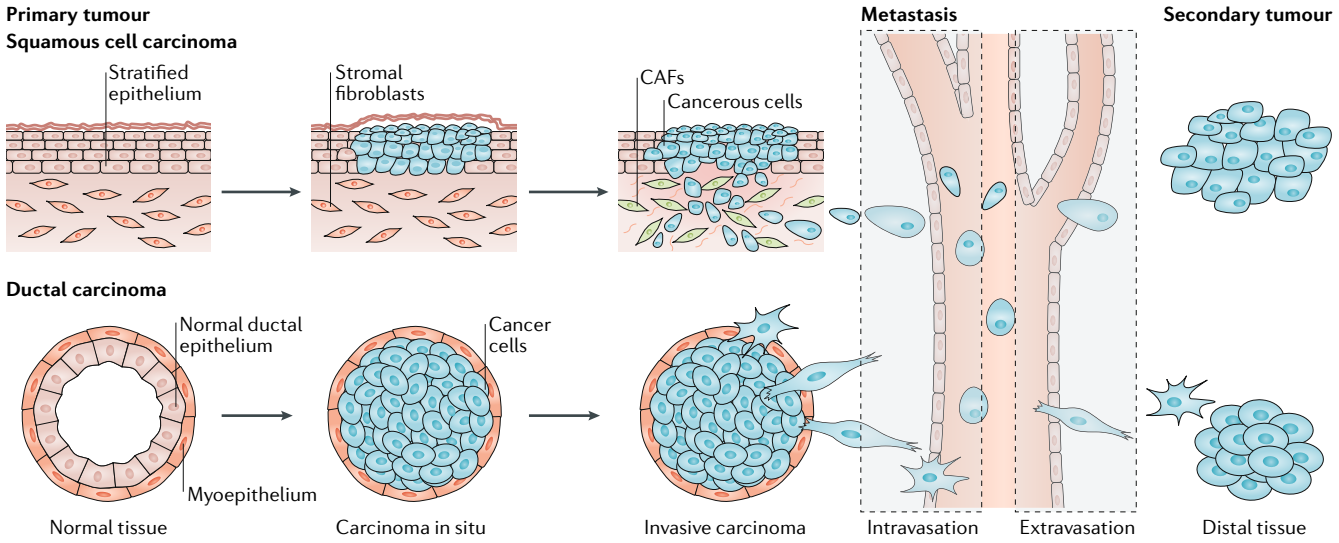
Primary tumour
The mutation of a single cell leads to uncontrolled division, resulting in an excess of abnormal cells. As the mass grows, the cells can acquire additional mutations and remodel the surrounding tissue, forming a primary tumour. Tumours are heterogeneous and often lack the polarity and cellular organization of the original tissue.

Epithelial-to-mesenchymal transition
Epithelial-to-mesenchymal transition (EMT) is a cellular programme that causes cells within a primary tumour to lose characteristic cell–cell adhesions, to break the basement membrane associated with an epithelial phenotype, to transition to a mesenchymal phenotype that lacks cell polarity and to upregulate and/or activate specific transcription factors, such as Twist family bHLH transcription factor 1 (TWIST1). The EMT programme enables cells of the primary tumour to locally invade the surrounding stroma and is characterized by a shape change of the cells in the primary tumour.

Intravasation
Intravasation is the migration of cancer cells from tumour-adjacent stroma into a blood or lymphatic vessel. This is a multistep process, during which metastatic tumour cells migrate through the extracellular matrix and between cells in the vessel as well as through the water-tight junctions between endothelial cells to reach the fluid in the lumen of the vessel.

Extravasation
Extravasation is the exit of cancer cells from a blood or lymphatic vessel through the endothelial cell layer lining the vessel and into a secondary site distant from the primary tumour. This is also a multistep process, during which circulating tumour cells slow down and stop along the vessel wall through adhesion to endothelial cells. Cells break through the water-tight junctions between endothelial cells and the matrix within the vessel to invade new tissue.

Secondary tumour
A malignant tumour that grows in a secondary organ from cells originating from a primary tumour.



CAF, cancer-associated fibroblast.

still under intense investigation, and much remains to be understood.

In this Review, we discuss how biomaterials can be applied to model tumours and their microenvironments in vitro. We examine different materials that can be used to capture and measure cancer cells for diagnostics and prognostics and investigate biomaterials for their potential to be used for cancer treatment in vivo.

First attempts to model the tumour ECM

Matrigel

The discovery of dynamic reciprocity was made possible, in part, through the use of tissue-derived biomaterials, which mimic an in vivo microenvironment for in vitro studies of cell–ECM interactions. Matrigel is a solubilized, gelatinous protein mixture composed of reconstituted basement membrane, which was originally isolated from Engelbreth–Holm–Swarm (EHS) mouse sarcoma cells[8,9] and is still routinely used to support the formation of epithelial structures. Matrigel mainly consists of assorted ECM proteins such as laminin, type IV collagen, heparin sulfate proteoglycans and entactin. However, Matrigel also contains growth factors that can potentially interfere with cell signalling events and thus affect the interpretation of results[10]. Therefore, growth factor-reduced versions of Matrigel have been developed to enable 3D cell culture characterization that focuses on the material properties alone. The use of Matrigel partly allows for the in vitro recreation of the architectural and biochemical complexity of an in vivo cell microenvironment. For example, the first 3D culture of primary mammary epithelial cells (MECs) was achieved using Matrigel, demonstrating that the basement membrane plays a crucial role for the 3D organization of MECs and for the generation of stable and functional hollow-lumen acinar structures[11]. 3D culture of MECs using Matrigel allows the cells to aggregate, remodel the ECM and self-organize into a layer of polarized cells — often with a hollow lumen — through the establishment of epithelial junctions and polarity. This approach enabled the first in vitro differentiated functional alveolar organoid, paving the

Box 2 | **Key aspects of biomaterials for cancer biology**

Biomaterial
A natural or synthetic substance that is compatible with biological systems. It can be engineered for research, diagnostic or therapeutic purposes.

Hydrogel
A polymer gel in which natural or synthetic hydrophilic polymers can be physically or chemically crosslinked to produce a hydrogel that contains different volume fractions of water. The physical and chemical properties of hydrogels can be modulated, for example, by altering the crosslink density or bulk polymer concentration to increase stiffness or by adding peptides or degradation enzymes.

Stiffness
The resistance of a material to deflection or deformation in response to an applied force. Stiffness is a term synonymously used in the biological literature for Young's modulus or elasticity. The stiffness of tumour tissue is higher than that of healthy stromal tissue, leading to alterations of mechanosignalling pathways in cancer cells. Therefore, it is important to model the correct stiffness of tumour tissue in vitro to recreate relevant biomaterial-based cancer models. The stiffness of tissue culture plastic (GPa) is orders of magnitude higher than that of human tissues (kPa), and the stiffness of tumours and of their adjacent stroma is usually an order of magnitude higher than that of healthy tissues; for example, the stiffness of mammary tumours is ~5 kPa, and the stiffness of adjacent stroma is ~0.1 kPa (REF.[3]).

Topography
A parameter that corresponds to the shape and features of the surface of materials. The topography changes with the architecture of the extracellular matrix (ECM). For example, hydrogels and fibrillar matrices have generally smooth and rough topographies, respectively. Increasing collagen deposition increases migration and invasion of tumour cells up to the point at which pore size becomes the limiting factor.

Porosity
Porous or empty spaces within a material are formed as a result of polymer crosslinking. In hydrogels and fibrillar matrices, pores are filled with fluid, and tumour cells can migrate through them to invade the material. The minimum size limitation for cells to pass through pores is <5 μm^2 (REF.[23]); however, cancer cells can release matrix-cleaving enzymes to degrade the ECM and make room to migrate, which can be recreated in biomaterials using enzyme-degradable peptides as crosslinkers.

way for morphogenesis and developmental studies in vitro using biomaterials. These recombinant basement membrane-derived systems have also been used to assess differences in gene expression profiles between cell lines[12]. The use of Matrigel in combination with collagen further enabled the identification of cellular differences between normal and malignant cells in 3D[13].

The seed and soil hypothesis of metastasis

Originally, biomaterials were mainly used to understand how the adjacent tumour ECM regulates tumorigenesis. An equally important aspect — albeit less well studied — is the cellular and ECM composition of the microenvironment at distant sites of metastasis. The distant microenvironment was described by Stephen Paget as the 'soil' in his 'seed and soil hypothesis'[14]. On the basis of the analysis of the data of a large cohort of patients with breast cancer, he hypothesized that the microenvironment plays a crucial role in regulating the seeding and growing of secondary tumours. Similar to disease progression-associated changes of the tumour ECM, Paget suggested that unique features of the soil can cause cancer cells to metastasize to specific locations. Stromal and immune cells are also part of the soil, migrating to distal sites prior to the arrival of tumour seeds[15]. Extracted stromal ECM components can further promote or prevent tumour progression[16,17], demonstrating that the ECM plays a role in seed implantation and can remodel tumour stroma[18]. Both in the tumour microenvironment and at distant sites of metastasis, a complex network of ECM proteins contributes to tumour progression and impacts cancer cell behaviour. Natural biomaterials can be applied to recreate these microenvironments, incorporating different stromal and ECM features to improve in vitro disease models and to develop new generations of therapeutics and diagnostics. However, there is a veritable balance between preserving the native ECM structure and composition to precisely resemble the in vivo architecture and the removal of cellular and antigenic material, such as nucleic acids, membrane lipids and cytosolic proteins, to be able to reproducibly use these biomaterials in vitro. These caveats have led to the development of new natural matrices as well as synthetic hydrogels that are more reductionist than these initially used natural biomaterials.

Engineering the tumour microenvironment

Natural biomaterials

Mimicking the microenvironment of tumours requires the use of 3D rather than 2D architectures to enable morphogenesis. Collagen gels were first used as 3D scaffolds to demonstrate how normal murine MECs form lumens in 3D as opposed to monolayers on 2D substrates[7], emphasizing the importance of 3D materials to recreate in vivo cell morphologies in vitro. The first ECM-specific behaviour observed using 3D biomaterials was cancer cell dissemination from tumour cell aggregates. In collagen gels, mammary carcinoma cells migrate as single cells with larger protrusions and higher local dissemination than cells embedded in Matrigel, in which cells migrate in a collective pattern[19]. These data indicate that protein composition of the matrix is an important property of neoplastic cell invasion. Unlike invasive carcinomas, malignant cells establish a vasculogenic network when embedded in collagen matrices with small pores and short fibres; tumours that feature such a tumour-adjacent matrix are correlated with poor prognosis. Such a short fibre-based network is not established if cells are exposed to increasing amounts of recombinant basement membrane[20], and thus the vasculogenic network is not formed. This effect can be titrated, and increasing collagen concentration restores vascular network formation[21].

Natural matrices containing collagen and/or recombinant basement membrane can be crosslinked or fabricated at different concentrations to modulate their stiffness and thus enable the assessment of the influence of stiffness in concert with specific genetic alterations. For example, MECs respond to increasing collagen matrix stiffness, which is achieved through adding collagen proteins, by breaking the acinar structure and invading into the ECM. If the genome of the MECs contains specific cancer-driving oncogenes, for example, receptor tyrosine-protein kinase ERBB2 (REF.[5]), they display an even more aggressive phenotype when interacting with a stiff matrix. MicroRNAs also play a role in regulating the expression of genes that favour tumour progression and are implicated in the increased stiffness sensitivity of MECs[22]. In addition to stiffness, ECM porosity further plays a central role in cancer cell migration and tumour

growth. Small pore sizes reduce the migration speed of cells in natural ECMs, such as collagen, by acting as barriers for nuclei deformation. A similar behaviour has been observed using synthetic materials[23]. However, in contrast to synthetic materials, cells can use matrix metalloproteinases (MMPs) to degrade natural ECM and increase the pore size to migrate through dense collagen gels[24]. Beyond a specific pore size threshold, myosin-mediated traction forces can propel the nucleus forward and allow migration through a dense ECM[25,26]. These data indicate that ECM fibre assembly, porosity and composition affect ECM architecture and material properties and, consequently, cancer cell migration and dissemination. However, in a natural matrix, the biochemical and biophysical parameters of the ECM cannot be decoupled; that is, individual matrix properties can only be varied relative to each other. This makes it challenging to accurately predict the impact of individual effects of natural ECMs on cell migration[27,28]. For example, altering ECM stiffness by adding more matrix protein also affects the adhesive properties of the matrix[29]. Moreover, batch-to-batch variations can influence the reproducibility of experiments; even in commercial products, such as Matrigel, variation in matrix protein composition, for example, fibronectin, can drive differences in cell behaviour[30]. Therefore, although natural ECM mimics the microenvironment of native tissue very well, coupled variation of ECM parameters and inconsistent composition are valid concerns.

Given these issues, a clear consensus on the relationship of migration and ECM parameters has not yet been achieved. For example, the concentration of specific ECM components has been shown to have either biphasic[31] or direct[32] effects on cancer cell migration. Cell contractility is also required for migration along a matrix, but how specific ECM properties guide cell contractility is still under debate. In collagen matrices, the forces generated by mammary carcinoma cells are independent of collagen concentration and matrix stiffness[33]. However, invasive cancer cells, which transition to a more mesenchymal phenotype with a spindle-like morphology, exhibit more processive or directed migration, making them more invasive with increasing collagen concentration[34].

These (sometimes controversial) observations have also been made using pristine natural matrices made from recombinant or animal-derived proteins. A better suitable ECM model is matrix exposed to clinically relevant doses of radiation. Irradiated matrices exhibit altered structures that substantially reduce metastatic cancer cell adhesion, spreading and migration[35]. In addition to the interest in using more relevant and reductionist materials, there is an equal interest in moving from common cell lines to their primary human tumour cell counterparts owing to their different and potentially more relevant behaviours. Together, this has created the push to move to mainly synthetic material systems.

Synthetic biomaterials

Natural materials have been key for initial investigations of ECM and cancer, but owing to their above-mentioned disadvantages, synthetic materials are increasingly used to mimic tumour ECM (FIG. 1). Synthetic materials have the advantage that parameters can be decoupled[36]; tuning one parameter, such as substrate stiffness, does not affect other parameters, such as fibre architecture or pore size[37] (BOX 2). They can also serve as a platform for cell adhesion by providing different ECM proteins or peptides, such as arginine–glycine–aspartic acid (RGD), glycine–phenylalanine–hydroxyproline–glycine–glutamate–arginine (GFOGER) or isoleucine–lysine–valine–alanine–valine (IKVAV), to understand how specific ECM components regulate tumorigenesis (FIG. 1a). For example, polyethylene glycol chains decorated with peptides of laminin 1 and type I collagen, but not of fibronectin, support invasive behaviours of metastatic prostate cancer cells, which is not observed for non-metastatic cancer cell lines[38]. Therefore, such systems can be potentially used to separate neoplastic cells from a mixed cell population. Synthetic materials can be easily functionalized with not only adhesive ligands but also a variety of other signalling proteins and peptides; for example, materials can be crosslinked with protease-degradable linkers, thus allowing the cells to control local matrix properties in a similar way as in natural matrices[39]. However, synthetic materials enable variation and individual control of ECM properties, although the combination of specific properties or proteins does not necessarily result in a linear cell response[30,40]. For example, cancer cells show different sensitivity to combinations of matrix proteins than to the individual proteins[41] and can be more or less responsive to specific matrix properties if they adhere to more or less permissive matrix proteins[30].

Modulating matrix stiffness. A breast tumour mass is routinely identified by manual palpation; the patient or doctor identifies a stiff lump relative to the compliant surrounding tissue. In epithelial tumours, a direct correlation between stiffness and metastatic potential has been reported[3,5,42–45]; however, this correlation has not been observed in all animal models[46]. To tune stiffness in natural ECMs, matrix concentration is increased, which also affects porosity and ligand density[3]. By contrast, in synthetic materials, changing crosslink density or bulk polymer concentration allows for the variation of stiffness by several orders of magnitude without modifying adhesion ligand density[47] (FIG. 1b). Most epithelial tumour models use a combination of naturally derived or natural and synthetic matrices in 3D[48,49]. These approaches using materials with increasing stiffness have been applied to study the mechano sensitivity of mammary epithelia during their transition to a mesenchymal phenotype, that is, the epithelial-to-mesenchymal transition (EMT). A stiff matrix triggers focal adhesion assembly through stress-induced elastic deformation, which in combination with cell contractility activates extracellular signal-regulated kinase (ERK) and the RHO family of GTPases, driving MECs towards EMT[3] (FIG. 2). Increasing matrix stiffness also triggers the release of the EMT transcription factor Twist family bHLH transcription factor 1 (TWIST1) from its cytoplasmic binding partner RAS GTPase-activating protein-binding protein 2 (G3BP2), its translocation to the nucleus and initiation of an EMT transcription programme[45].

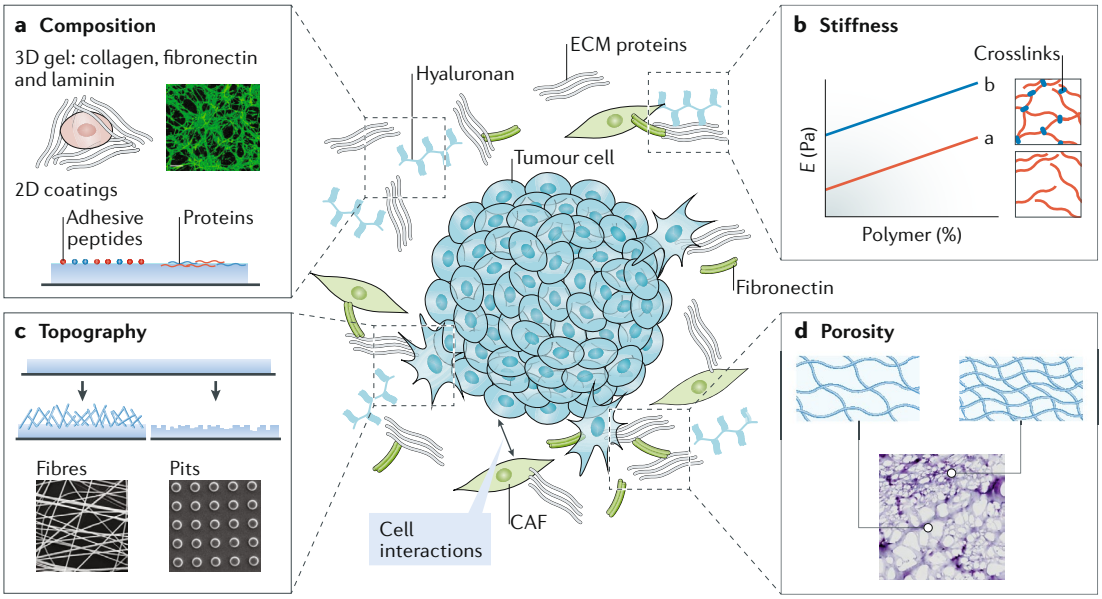


Fig. 1 | **Modelling the tumour microenvironment.** The tumour microenvironment constitutes the niche that surrounds a tumour, including extracellular matrix (ECM), cells and signalling molecules. The niche is characterized by specific dynamic ECM properties. **a** | The composition of the ECM can vary in terms of both ligand type and ligand presentation. 3D hydrogels made of ECM proteins or 2D materials can be used to recreate a specific ECM composition. The ligand type[41] and concentration[3] affect cell behaviour and can induce an epithelial-to-mesenchymal transition (EMT). **b** | Stiffness, that is, the Young's modulus, can also impact EMT[3,45]. The Young's modulus (*E*) of a material can be modified by changing chain entanglements (line a) or crosslinking (line b). The stiffness is measured as the force per cross-sectional area of the material. **c** | Topographical features of the niche can be recreated by spinning polymers into fibres and depositing them as a thin layer on a surface, to which a cell can adhere. Alternatively, a material can be etched to create specific nanotopographical or microtopographical features, such as pits. Such topographies can be used to induce cell transformations[58–62] or to capture cancer cells[82,85,86]. **d** | The pore size and pore connectivity of the tumour microenvironment can be modelled by modulating bulk polymer density or droplet size in emulsions. Non-malignant cells are highly sensitive to pore size[63,64]; materials with small pores can inhibit migration and proliferation, and large pores are felt by the cells as 2D surfaces. CAF, cancer-associated fibroblast.

Additional evidence suggests that hydrogel stiffness regulates not only malignant transformation but also dissemination and migration of invading cancer cells[50]. Metastatic cells have tumour-specific stiffness preferences; at an optimal stiffness, corresponding to the stiffness of a specific tumour type, they express markers consistent with highly migratory cells and migrate faster than at sub-optimal stiffness[51].

Synthetic materials can also be designed as dynamic systems, in which crosslinking can be gradually[52,53] changed or modified on demand[44,54,55], thus better mimicking slow disease progression. Collective cancer cell behaviours can be substantially different in materials that stiffen following polarization than in materials with static stiffness[56]. Controlled degradation[57] can also provide a strategy to examine cell behaviour in response to an environment that becomes increasingly softer and to identify mechanotransduction pathways that can slow tumorigenesis. Therefore, matrix stiffness and the timing of its presentation are important ECM properties that influence neoplastic cell behaviour.

Fibre architecture, topography and porosity. The architecture and topography of ECM fibres also affect the behaviour of neoplastic cells. Cancer cells can sense whether the surface is atomically flat or has a roughened topography (FIG. 1c), which can induce invasion and metastasis. For example, fibrillar matrix structures can be synthetically recreated using electrospun fibres, such as silk, to support 3D cell migration of both malignant and non-malignant cell lines[58,59]. Alternatively, polydimethylsiloxane (PDMS) is a commonly used polymer for topographical studies. Using patterned PDMS substrates, it has been shown that neoplastic cells are less sensitive to geometrical cues than non-malignant cells[60,61]. On micrografted surfaces, MECs enter a dormant state, whereas their neoplastic counterparts continue to proliferate through a RHO–RHO-associated protein kinase (ROCK)–myosin-dependent pathway[62]. This principle also extends to other roughened surfaces, on which malignant cells appear less sensitive and continue to grow and migrate independent of roughness[60,62].

Similarly, ECM porosity, which dictates cell spreading, can differentially affect non-malignant and metastatic cells (FIG. 1d). For example, metastatic cells can migrate through PDMS channels that are smaller than the diameter of their nuclei by breaking and reforming their nuclear envelope[23]. 3D material systems containing collagen and agarose can be used to independently modulate stiffness, porosity and ligand density. If the porosity of the material is decreased independent of other properties, glioblastoma cell migration is sterically hindered[63]. Conversely, non-malignant cells sense porosity together with other properties, such as stiffness;

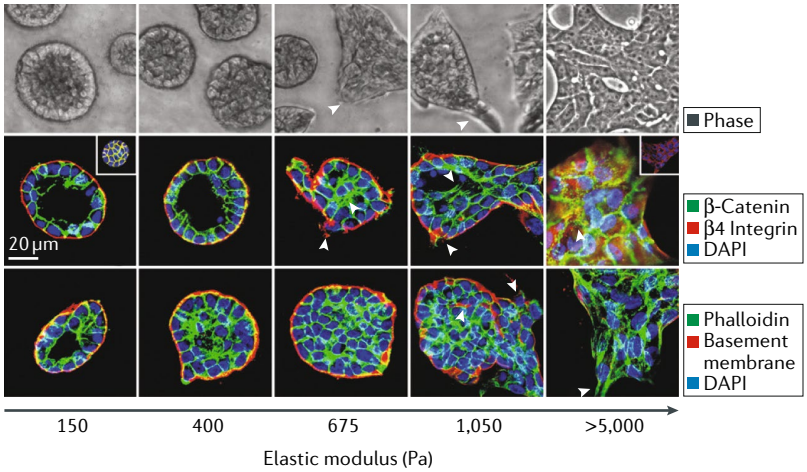


Fig. 2 | **Matrix stiffness regulates the epithelial-to-mesenchymal transition.** Phase contrast and fluorescent images of mammary epithelial cell colonies on polyacrylamide hydrogels of indicated stiffness (150–5,000 Pa) with Matrigel overlay are shown. Microscopy images show colony morphology after 20 days. The fluorescent images show β-catenin (green) before and after (inset) triton extraction, β4 integrin (red), epithelial cadherin (E-cadherin) (red; inset) and nuclei (blue). In the bottom images, actin (green), laminin 5 (basement membrane; red) and nuclei (blue) are shown. DAPI, 4′,6-diamidino-2-phenylindole. Figure is reproduced with permission from REF.[3], Elsevier.

for example, in channels of decreasing width, the migration speed of non-malignant cells increases with stiffer channel walls[64]. These data suggest complex and often coupled interactions and therefore do not yet allow an overarching conclusion or propose the ideal material for modelling the tumour microenvironment. However, individual ECM properties have already been identified that can be modulated using biomaterials to study their effects on cancer cells (TABLE 1).

Model requirements beyond materials

Tumours are often described as organs that contain different cell types, including CAFs[65], endothelial cells, pericytes and immune inflammatory cells[66]. The vast majority of biomaterial-based models are incomplete because they do not incorporate these important cell types that modify the microenvironment. Cancer cells secrete soluble factors that activate CAFs, leading to a change in CAF protein expression and an increase in MMP secretion and CAF contractility[67–69]. CAF-generated forces promote angiogenesis[70] and generate holes in the matrix to facilitate cell invasion[69]. CAFs can also directly bind to cancer cells through heterotypic epithelial cadherin (E-cadherin; also known as CDH1) and neural cadherin (N-cadherin; also known as CDH2) junctions and pull cancer cells away from the tumour[71]. CAF contractility further promotes the nuclear translocation of Yes-associated protein YAP65 homologue (YAP1), which in turn results in matrix stiffening, angiogenesis and cancer cell invasion. This positive feedback loop drives tumour progression[72]. However, most current biomaterial approaches to the niche lack these important interactions and signalling events.

Metastasis of cancer cells further depends on the ability of cancer cells to migrate through the stroma, intravasate blood vessels, survive in the circulation and extravasate into new matrix to colonize distant tissues (BOX 1). Although no hydrogel system to date mimics all these stages, materials-based microphysiological systems have been explored to mimic specific steps in this process, such as extravasation, in which cancer cells pass through the endothelium; for example, microphysiological systems can be fabricated using PDMS to engineer a perfusable microvascular network with hydrogel regions and media channels. Such systems are thin and composed of neo-vessels, allowing imaging analysis to study transendothelial migration[73]. By applying this in vitro approach, it has been shown that tumour necrosis factor (TNF)-α increases endothelial cell permeability, facilitates tumour cell intravasation[74] and modulates extravasation[75,76]. Microphysiological systems can also be used to investigate metastasis of certain cancer cells to specific secondary sites. For example, a microenvironment containing osteoblasts can be used to elucidate why breast cancer cells preferentially metastasize to bone. A higher number of breast cancer cells extravasate into the bone cell-conditioned microenvironment than into a collagen matrix, suggesting that bone-secreted chemokines such as CXC-chemokine ligand 5 (CXCL5) play a role in the chemotactic migration of breast cancer cells[77]. These systems enable the investigation of the contribution of specific families of cell-secreted cytokines to cancer cell metastasis, which is difficult to dissect in animal models. Further development of microfluidic devices and incorporation of various materials will make in vitro models increasingly relevant for cancer biologists as reductionist systems to recreate more steps of the metastatic process within one system.

Capturing cells in blood and stroma

Biomaterials can be applied for diagnostic and prognostic screening of cancer in vivo and ex vivo (FIG. 3). The current standard of care primarily consists of regular screenings, such as mammograms for breast cancer, flexible sigmoidoscopy or faecal occult blood test for colorectal cancer[78] and computed tomography and chest radiography scans for lung cancer[79]. However, by the time the disease is observable, the tumour has often already metastasized. To detect tumours in patients earlier and more accurately, biopsy samples can be taken and genetically tested for prognostic markers, for example, breast cancer markers breast cancer type 1 susceptibility protein (BRCA1) and ERBB2 by using mRNA microarrays[80]. Such assays have dramatically reduced cancer occurrence; however, they do not directly detect disease-causing cells.

Biomaterial-based technologies have primarily focused on capturing circulating tumour cells (CTCs). CTCs are a small fraction of cells that disseminate from primary tumours and are thought to be responsible for the haematogenic spread of cancer to distant sites[81,82]. Increased CTC levels in the blood are correlated with negative prognosis. Therefore, CTC isolation and quantification are essential for the early detection of metastasis and subsequent treatment[82]. However, CTCs are difficult to isolate with high efficiency and purity[81] and thus their unique molecular signatures remain elusive[82]. The most commonly used CTC isolation method relies on increased epithelial cell adhesion molecule (EPCAM)

Table 1 | **Biomaterials for modelling the tumour microenvironment**

Biomaterial	Model	Advantages	Disadvantages	Refs
Synthetic material				
Polydimethylsiloxane (PDMS)	2D micropatterns	Flexible substrate with patterns promoting cancer cell alignment	Uncertain viscoelastic mechanics and protein attachment	37,148
	Microchannels and microfluidics	• Directed cell migration in channels • Cell confinement • Confined fluid flow for controlled application of shear stress to cells	• Curing ratios often create materials that are less flexible • Static substrate	23,114
Polyethylene glycol diacrylate (PEGDA)	3D culture	• Wide stiffness range • Direct conjugation of many types of adhesive ligands • Can be used to identify tumour-specific stiffness	–	51
Polyethylene glycol (PEG)	3D culture	• Wide stiffness range • Direct conjugation of many types of adhesive ligands or degradable linkers • Inert and biocompatible	Backbone is not degradable	39,149,150
Poly(lactide-co-glycolide) acid (PLGA)	3D culture	• Porous scaffold • Biocompatible • Biodegradable	Methyl side groups increase hydrophobicity	151,152
	Implantable material	Recruitment and capture of metastatic cells	Degradation prior to cell capture	93
Poly(ε-caprolactone) (PCL)	Implantable material	Recruitment and capture of metastatic and immune cells	Degradation prior to cell capture	92
Polyacrylamide	Substrate gradients	• Sequential polymerization to create spatial patterns • Indication of metastatic cell 'memory' • Small well polymerization for high-throughput drug screens	Substrate stiffness does not change with time	153–155
	Used with Matrigel overlay for 3D culture	• Wide stiffness range • Conjugation of individual or multiple ligands resulting in nonlinear cell responses	• Cytotoxic prior to polymerization, preventing cell encapsulation • Difficult to measure forces in 3D	3,30,40,45
	Elastic 2D substrate	Measurement of traction forces in cancer cells	• Cytotoxic prior to polymerization, preventing cell encapsulation • Difficult to measure forces in 3D	42
Synthetic–natural hybrid materials				
Polyethylene glycol-heparin	3D culture	• Direct conjugation of adhesive ligands • Enzymatically degradable	Limited degradation control	38
Methacrylated hyaluronic acid (MeHA)	3D culture	• Direct conjugation of adhesive ligands • Enzymatically degradable • Temporal gradients through sequential crosslinking	• Radical polymerization limits in vivo application • Can induce DNA damage • Modifications can reduce bioactivity	44,54,55
Natural materials				
Matrigel	3D culture	• Established fibrillar model system • Temperature-based polymerization • Easy encapsulation methods • Growth factor-reduced version • 3D organization of acinar structures	• Batch-to-batch variation • Difficult to independently modulate parameters • Tumour-derived (inductive composition) • Temperature sensitive	8,9,11,30
Alginate	3D culture	• Stiffness can be modulated independently of architecture • Time-dependent stiffening with calcium crosslinking • Enables mammary epithelial cells to polarize before EMT	Calcium-dependent covalent bonds	56,156
Type I collagen	3D culture	• Fibrillar • Adhesion of multiple cell types • Facilitates cell invasion • Shows same radiation damage as tumours	• Transglutaminases and oxidases can crosslink with limited range Harsh organics are more common crosslinkers with a wider range • Limited stiffness range of ~1–1,000 Pa	23,35,69,157
	Matrigel-impregnated	Migration is biphasic and directly dependent on concentration	• Pore size changes with Matrigel concentration • Limited ligand presentation	31,32
	Agarose-impregnated	• Stiffness can be modulated independently of ligand density • Restricted invasion of glioma cells	Pore size changes with agarose concentration	63

EMT, epithelial-to-mesenchymal transition.

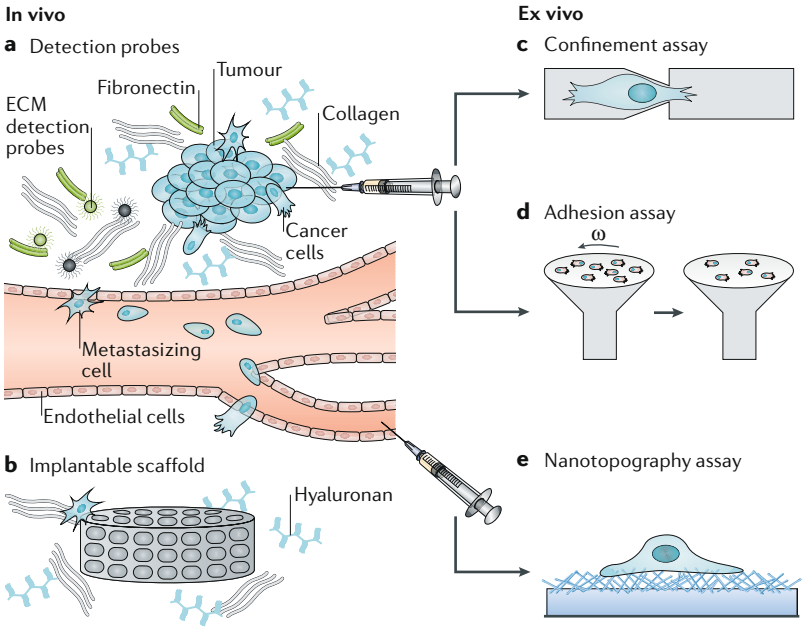


Fig. 3 | **Next-generation material-based cancer technologies.** The specific interactions between cancer cells and the tumour stroma can be exploited for the detection of cancer cells. **a |** Magnetic resonance imaging (MRI) or positron emission tomography (PET) contrast agents can be conjugated with extracellular matrix (ECM)-affinity peptides to create specific probes to target the dense ECM of the tumour stroma for the detection of mature tumours in vivo. **b |** Implantable scaffolds can be used to recreate a pre-metastatic niche at the implant site, recruiting cells for capture and therapy and at the same time lowering the tumour burden in typical secondary metastasis sites. **c,d |** Confinement assays or adhesion assays can be applied to test cells obtained from tumour biopsy samples for their aggressiveness by measuring cellular deformation or adhesion to specific ECM molecules. Omega (ω) is the angular velocity that defines the shear stress applied to cells. **e |** Circulating tumour cells (CTCs) can be isolated from patient blood samples using nanotopography assays that take advantage of the affinity of CTCs for nano-roughed substrates.

expression on the surface of CTCs[81], which is used by the US Food and Drug Administration (FDA)-approved CellSearch System. However, this system requires a very large sample volume, has low sensitivity and is time consuming[81].

Ex vivo detection using nanotopographies

CTC capture efficiency can be improved by increasing the local concentration of capture substrate or by coupling the substrate with surface-functionalizing molecules, such as antibodies or aptamers. For example, microfluidic chip assays composed of PDMS microposts with a surface coating of anti-EPCAM antibody can concentrate CTCs in smaller sample volumes[79] than systems without antibody coating. Silicon nanopillars further improve CTC capture by clustering antibodies through binding to streptavidin or gold[83]. Aptamer-functionalized gold nanopillar arrays show efficient cell release through cleavage of the sulfur–gold bonds between the aptamers and the gold nanopillars[84].

CTC purification and capture can also be achieved using artificial nanoscale topographies, mimicking structural features and dimensions of ECM[81]. Cancer cells preferentially adhere to nanostructured rough substrates compared with smooth substrates, even in the absence of surface functionalization with antibodies[82]. For example, fractal nanostructures have an uneven topography and a crystalline structure, which increase cancer cell binding to the surface[85,86]. Fractal nanostructures can be generated from synthetic materials, such as TiO_2, with inverse opal photonic crystals to mimic cellular components or natural materials, such as hydroxyapatite nanostructures of seashells[86,87]. Alternatively, rough nanoscale substrates can be fabricated with an anti-EPCAM antibody-coated, mesh-like silicon nanowire substrate and overlaid with a PDMS-based chaotic mixer[88,89]. These systems show a >95% capture efficiency of EPCAM-positive MCF7 breast cancer cells, which is more than 20-fold higher than EPCAM antibody-coated smooth substrates[90,91]. The addition of electrospun thermoresponsive nanofibres enables an even higher capture efficiency and allows on-demand release and single-CTC analysis, for example, for next-generation sequencing[88]. Cell release can also be achieved by using degradable zinc-phosphate nanosubstrates[92].

Nanostructured surfaces enable high capture efficiency but cannot provide high cell purity owing to nonspecific cell adhesion. Dual-functional lipid coating can be applied to improve the capture specificity of nanopillars owing to the higher concentration of antibody on the surface and inhibition of nonspecific cell adhesion[93]. Poly(carboxybetaine methacrylate) brushes also decrease nonspecific cell adhesion, and the active carboxyl groups capture CTC-specific biomolecules[94]. These nanostructure-based methods enable ex vivo detection of CTCs, demonstrating how specific ECM properties, such as topography, can be exploited to increase capture efficiency and provide a strategy for proactive disease monitoring. It has been suggested that CTC detection probability scales with patient mortality[79] and, thus, technologies for the continuous detection of CTCs could provide a strategy to detect cancer cell metastasis early enough to substantially increase patient survival.

In vivo cell detection using implantable materials

Biomaterials can also be implanted to monitor tumour progression in vivo[95,96]. According to Paget's seed and soil hypothesis, secondary metastases do not occur randomly[14]. Specific microenvironments are primed for tumour cell colonization through the presence of tumour-supportive fibroblasts, endothelial progenitor cells, immune cell-secreted factors and ECM-remodelling events[95–97]. Current imaging techniques are limited in their ability to detect micrometastases that form at distal sites[95–97], which reduces their prognostic capabilities and offers an area of opportunity for biomaterial-based solutions.

For example, microporous scaffolds such as poly(lactide-co-glycolide) acid (PLGA) can be implanted to recruit and capture metastasized cells. Breast cancer cells that have metastasized to the brain can be injected into the fat pads of mice and entrapped in an implanted PLGA scaffold. Mice with scaffolds implanted to capture circulating cells develop fewer lung tumours[96] than animals without any implanted material, indicating that the scaffolds reduce secondary metastases formation. Poly(ε-caprolactone) (PCL) has similar physical properties to PLGA but degrades more slowly[95]. PCL scaffolds

can also be used to recruit tumour and immune cells, which are implicated in establishing a pre-metastatic niche, and to decrease the number of detectable tumour cells in common secondary sites[95,98–100]. Additional modifications, such as graphene oxide (GO) functionalization, can further increase cancer cell adhesion compared with non-functionalized scaffolds[101]. GO addition to the scaffold can also enable photothermal ablation of cancer cells within the scaffold owing to the near-infrared absorbance of GO[101,102], demonstrating how implantable scaffolds can be used for both cancer cell capture and therapy. Besides chemical modifications, scaffolds can also be coated with ECM proteins, including fibronectin and type IV collagen, to improve scaffold capture efficiency. Each tumour type is characterized by specific ECM combinations and thus scaffolds can be coated with a tumour-specific ECM that supports metastases[41] to improve cancer cell recruitment. For example, coating with decellularized lung or liver matrix of metastatic tumours substantially increases capture efficiency[97].

Matrix is not the only niche component that can be used to improve cell capture. Cancer cell-secreted exosomes or haptoglobin can also be incorporated into synthetic scaffolds to create a bioengineered niche that captures metastatic cells more effectively than tissues to which cells commonly metastasize and increases survival in animals implanted with these scaffolds[103,104]. Natural materials such as silk can also be functionalized with proteins, such as bone morphogenetic protein 2 (BMP2), to mimic a bone marrow microenvironment. This material can serve as a surrogate for a pre-metastatic niche and recruit metastasizing cancer cells that would normally home to bone marrow[105]. In particular, BMP2 increases the adhesion of metastatic prostate and breast cancer cell lines to the scaffold[105,106]. Such scaffolds can be implanted to capture tumour cells, reduce the tumour burden on standard metastatic organs and prevent the local remodelling of tissue into a pre-metastatic niche, making them potent therapeutic tools to detect, capture and ablate metastasized cancer cells. However, these scaffolds do not have an inherent proclivity to capture specific cell types.

Ex vivo cell detection using physical properties

Cells migrate through the stromal ECM through confined pores, which can be smaller than the nucleus of the cell. To achieve this, cells can either degrade adjacent matrix using MMPs[24] or physically deform it[107]. Increased MMP expression and decreased nuclear size[108,109] are associated with aggressive cancers and thus cell deformability is emerging as a marker for the invasive potential of cancer cells[110]. Assays for the investigation of cellular deformability exploit the variable pore size in the ECM to shed light on the relationship between the degree of deformation and the corresponding invasive and metastatic potential. The most common strategy is to micro-fabricate channels — for example, in PDMS — with defined geometries and track cellular movement. Cells with low expression of nuclear lamina proteins, which contribute to nuclear stiffness, pass more quickly through narrow regions[107] than cells with high lamin A and/or lamin C expression and stiff nuclei. Specific deformation tolerances can be assessed using funnel-shaped constrictions in series[111] or in parallel to analyse cell transition effects[112]. Metastatic cells modulate their morphology, as they are forced into confined spaces more than their non-metastatic counterparts, resulting in faster and larger deformation events[112]. Highly metastatic cells can even rupture and reassemble their nuclear envelops when they encounter transit constrictions[23]. Intravasation constitutes one of the most restrictive parts of the journey of a metastasizing cell. Microfluidic devices with cell and nutrient chambers separated by microchannels of varying width can be used to determine the minimum gap that cancer cells can migrate through in confined environments. Such a device has been applied to demonstrate that the nucleus is a crucial limiting factor for a cell to be able to traverse confined environments[113].

Constrictive devices rely on cell-generated forces; alternatively, external hydrodynamic forces can be applied to deform cells. Opposing flows, that is, hydrodynamic stretching, can uniformly deform cells, and the degree of deformation can be controlled by simply changing the flow rate[114] or through pinched-flow stretching in a single inlet[115]. The latter design forces cells to flow in the centre of the channel, siphons fluid on the sides of the channel away from the cells and then compresses the cells when the fluid is added back to the channel[115]. These assays can be applied to analyse cell deformability of single cells or populations of cells using pressure-driven microfiltration systems. Using these systems, it has been observed that induction of EMT or drug resistance leads to an increase in cell deformability[116]. Such microfiltration devices enable high-throughput assessment of transit time and deformability[117] to investigate a population of cells from a tumour. These assays, applying forces either internally or externally, measure internal features of the cytoskeleton that are found in metastatic but not in non-metastatic cells. Therefore, microchannel assays can be useful as diagnostic tools to assess the aggressiveness of cells isolated from tumour biopsy samples and to observe the effect of cancer therapies on cell deformability and thus disease progression.

Adhesion properties and mechanisms provide another physical metric to determine the metastatic potential of cancer cells. Assays that apply negative pressure to detach cells[118], to assess binding efficiencies to ECMs[119] or to analyse adhesion turnover[120] have demonstrated that adhesion is modulated differentially in metastatic cancer cells compared with in non-metastatic cells. For example, metastatic cancer cells can move rapidly through tissue through increased cation sensitivity that leads to more rapid formation and disassembly of focal adhesions than in their non-metastatic counterparts[121]. Cell–matrix adhesions are directly modulated by magnesium, manganese and calcium cations, which increase integrin affinities for matrix proteins in proportion to their concentration. The concentration of cations is tenfold lower in the stroma than in the tumour[122,123]. Thus, once metastatic cells reach the stroma, only cells with labile adhesions can migrate. Indeed, cancer cell adhesion strength to fibronectin and type I collagen at low cation conditions correlates with metastatic potential;

within a highly metastatic cell population, the subset of cells with high adhesion strength is less migratory and invasive than malignant and non-cancerous epithelial cells or strongly adherent metastatic cells[121]. Analysing the weakly adherent cell fraction enables the determination of the metastatic potential of a tumour in situ. Each of the above-discussed assays yields valuable information about the metastatic potential of cancer cells, which could make such devices useful diagnostic tools for prognostic assessment and for determining a course of treatment.

Non-invasive surveillance of tumour-adjacent stroma

Interaction with the surrounding matrix is an important regulator of cell dissemination, and various matrix properties can act as markers to detect and/or capture highly invasive cells that are predisposed towards tumour formation. Exploiting the similarities of tumour microenvironments across different cancer types opens up avenues for monitoring the presence and growth of primary tumours. For example, overexpression of integrins, common matrix signatures[41] and overexpression of specific MMPs can act as prognostic indicators of the metastatic potential of tumours in patients with primary breast tumours[124]. Unlike most physical parameters of the ECM, the composition of the tumour-adjacent stroma can be non-invasively monitored, making it an attractive property for the assessment of tumour progression in patients.

In addition to biochemical surveillance, imaging methods are also being explored using material-based probes. For example, a combination of high-affinity fibrin peptides and tracer molecules (that is, radioisotopes) that are detectable by magnetic resonance imaging (MRI) or single photon emission computed tomography (SPECT) are being developed to assess increased fibrin deposition in tumours[125,126]. Antigen-binding fragment (Fab) probes can be combined with a radioisotope to image fibrin clots in the tumour microenvironment[127]. Such probes also demonstrate low retention times in non-target tissue in vivo[126,128]. Fibronectin is also overexpressed during EMT, making it a prime target for early cancer detection probes[124,129–131]. Similar to MRI contrast agents for fibrin, gadolinium-based MRI contrast agents can be used to target fibronectin–fibrin complexes, demonstrating robust detection of the primary tumour and of >0.5 mm^3 micrometastases[129]. Most current strategies target major ECM components; however, probes that target more tumour-specific ECM elements, such as periostin in oesophageal cancer[132], could improve detection specificity, decrease background signalling through rapid clearance of non-bound contrast agents[124] and increase tissue penetration depth owing to their small size. These approaches, which are still being developed, enable us to image tumours with increasing spatial resolution, but they do not provide information about the aggressiveness of tumours.

Perspectives and conclusion

Strategies to understand and detect tumours have greatly improved our ability to recognize and assess specific tumour pathways and cell behaviours that are indicative of disease progression. As the field matures, cancer diagnosis and treatment will most certainly involve more materials-based approaches to address shortcomings in our ability to model, detect and treat cancer. Despite the development of a variety of dynamic, synthetic biomaterials applicable for the modelling and study of cancer, Matrigel is still most commonly used by cancer biologists for 3D cell culture systems even though it is highly variable, difficult to purify and derived from a mouse tumour. Therefore, the field of material science must continue to evolve and incorporate tuneable synthetic materials to help understand the cell behaviours induced by these increasingly complex materials.

As the biomaterials community, we also aim to clinically translate lessons learned from in vitro models to diagnostic assays. The substantial progress made in our understanding of the tumour as a material and in detecting and capturing cancer cells makes this an exciting time for material-based cancer research. There are great opportunities to improve our basic understanding of cancer and also our detection and treatment capabilities, for example, investigating tumour–stroma interactions in reductionist matrix systems, developing a complete tumour-in-a-dish model (including intravasation and extravasation) and understanding how animal models reflect clinical outcomes. Improvement of detection probes using biomaterials, whether invasive or not, is also a growing research area, which is reflected in the expanding body of literature. For example, during tumour growth, collagen, fibrin and hyaluronan concentrations increase in the surrounding ECM, and the matrix stiffens and is aligned by lysyl oxidases[5,133,134] to facilitate invasion[124,135]. Potential therapeutic avenues include the use of proteases to degrade matrix proteins and decrease stiffness to improve drug penetration. Conversely, hyaluronidase, which degrades the extracellular glycosaminoglycan hyaluronan, can be inhibited to limit tumour growth and metastasis[136,137]. Clinical trials of hyaluronidase delivery have demonstrated its safety[138], and a phase III study is currently being conducted (NCT02715804). Finally, future improvements in treatment options using biomaterials will ultimately impact clinical outcomes. For example, altering ECM structure could improve nanoparticle and drug delivery, resulting in more effective, deeper-penetrating therapies and improved patient outcomes[133,139–142]. In addition to enzymatic strategies, physical disruption of the matrix using high-intensity ultrasound can be used to improve the penetration of nanoparticles into the tumour tissue without damaging surrounding tissues[139]. Thermal strategies with nanotubes[143] or gold nanorods[144] can also be applied to denature the collagen matrix and increase tumour diffusivity.

Using biomaterials for the modelling, detection and treatment of cancer is a promising strategy. Another important contribution of material science in the near future will be to help rectify the differences in disease progression and treatment between humans, animal models and patient-derived xenografts[145]. Biomaterial-based models are reductionist in nature; thus, their application in vivo could improve the reliability of animal models, making them more predictive of patient outcomes[146]. Animal models are considered the standard assay for tumour biology, and material-based

in vivo strategies are required to understand the differences between humans and animal models. For example, recombinant, chemically defined natural[147] or synthetic[45] biomaterials could be used that can actively modify tissue properties[5]. Such materials have already enabled the identification of cancer stem cells and mechanotransduction mechanisms and have demonstrated how material properties can drive tumorigenesis, making future applications in vivo promising.

The examples discussed in this Review demonstrate that biomaterials can serve as powerful tools to replicate mechanisms of disease and the response to treatments in vitro. The materials-based strategies that have enabled these discoveries should be broadly applied in the future to further improve our understanding of cancer biology and to begin to impact clinical outcomes.

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Competing interests
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