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Review

Spatial -omics technologies: the new enterprise in 3D breast cancer models

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The fields of tissue bioengineering, -omics, and spatial biology are advancing rapidly, each offering the opportunity for a paradigm shift in breast cancer research. However, to date, collaboration between these fields has not reached its full potential. In this review, we describe the most recently generated 3D breast cancer models regarding the biomaterials and technological platforms employed. Additionally, their biological evaluation is reported, highlighting their advantages and limitations. Specifically, we focus on the most up-to-date -omics and spatial biology techniques, which can generate a deeper understanding of the biological relevance of bioengineered 3D breast cancer *in vitro* models, thus paving the way towards truly clinically relevant microphysiological systems, improved drug development success rates, and personalised medicine approaches.

Highlights

Advanced 3D breast cancer models offer a promising, clinically relevant *in vitro* tool for researchers. By closely mimicking the interaction between tumor cells and the extracellular matrix compartment, these models could be used to help screen drug efficacy and tailor personalised treatments.

Clinical translation remains challenging because these models are often biologically underevaluated and do not consider the models' diversity at the genomic, transcriptomic, proteomic, or epigenomic level in relation to matched patients.

Multiomics technologies applied to bioengineered 3D breast cancer models could be game changing to unravel the detailed mechanisms of interaction between cancer cells and stroma for each specific breast cancer subtype and to foster their validation and translation into clinics.

Great effort is being made in using spatial multiomics. However, the highly demanding costs of sequencing and informatic analysis are still bottlenecks that need to be overcome.

3D models as novel tools for cancer research

Historically, laboratory-based cancer research and drug development have employed the use of individual cancer cell lines in 2D cultures and animal models. Although essential for initial mechanistic studies [1], they lack clinical significance [2]. Recently, it has become clear that the interaction between heterogeneous cancer cells and the surrounding extracellular matrix (ECM) is crucial to properly study how tumor cells grow, invade, and metastasise to distant sites [3,4]. To better mimic the physiological cancer tissue microenvironment, the use of ECM-based 3D culture methods and related development of 3D tumor models have rapidly accelerated in recent years [5,6]. **Matrigel** (see Glossary) has been widely used as a 'gold standard' support matrix for 3D cell culture, thanks to its excellent biocompatibility for cell growth and proliferation [7]. Lately, increasing ethical and reproducibility concerns have prompted researchers to find alternative artificial ECMs (aECMs) that are more sustainable and of well-defined composition [8,9]. Different types of **biomaterials** and aECMs are being used, ranging from synthetic to natural sources [10,11]. Each biomaterial can be processed into specific constructs to fine-tune the biological, structural, and mechanical properties of the tumor of interest [12,13], aiming to accurately represent the native ECM composition and architecture [14,15]. Various processing and fabrication techniques are available for 3D *in vitro* tumor model development, which is discussed further in the following sections of this review.

Although these 3D models could serve as efficient *in vitro* tools to study the intricate interplay between cancer cells and their surrounding tumor microenvironment (TME), they still face some challenges and limitations. Standard endpoint analyses, such as imaging, can only provide a general understanding of the structural organization of the TME, using the expression of a handful of specific genes/proteins of interest. This greatly limits the characterization and biological validation of the developed 3D models, especially when trying to recreate the complex tissue

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architecture seen in humans. In fact, researchers have understood that detangling the mechanisms of interaction of defined cell types present in specific regions of the TME is vital to understanding cancer progression [16]. However, until recently, in-depth knowledge of the spatial distribution of highly multiplexed markers across a sample was not possible. The advent of spatial **multiomics** technologies is now making this possible, allowing the precise identification in the region of interest of a specific marker at **genomic**, **transcriptomic**, and **proteomic** levels and beyond. With the emergence of spatial multiomics technologies, it is now possible to better interrogate not just 3D models but also clinical breast cancer tissue specimens. Importantly, a better understanding of *in vivo* breast cancer cell organization will allow the continued development of yet more relevant 3D models and ultimately efficient anticancer treatments (Figure 1, Key figure). The ability to produce 3D breast cancer models at a fast and automated scale yields the possibility to rapidly test novel compounds for treatment [17,18]. Additionally, this fast turnaround time holds promise for personalised medicine approaches, whereby patient-specific treatments are tailored using patient-derived 3D models.

Thus, with the advent of more spatially elaborate bioengineered models comes the need for more spatially resolved endpoints. This review highlights the latest spatial multiomics platforms and how they can be applied to the bioengineered 3D cancer model field. Specifically, we focus our discussion on breast cancer, a challenging and highly histologically and molecularly heterogeneous type of cancer that represents a good candidate for these types of spatially resolved analyses. We provide a perspective on the latest developed 3D breast cancer models and how their detailed spatial characterization could bridge the gap between clinical relevance and bioengineered model validation.

Glossary

Biomaterials: natural or synthetic substances designed to interact with biological systems.
Bioprinting: the use of 3D printing technology with materials that incorporate viable living cells.
Genomic: the study of the complete DNA sequence of organisms.
Matrigel: a commercially available solubilised basement membrane matrix secreted by Engelbreth-Holm-Swarm mouse sarcoma cells.
Microfluidics: the science of manipulating and controlling fluids, usually in the range of microlitres (10^{-6}) to picolitres (10^{-12}), in networks of channels with dimensions from tens to hundreds of micrometres.
Multiomics: the simultaneous measurement and combination of two or more -omics data set modalities.
Proteomic: the large-scale study of the complete set of proteins expressed by an organism.
Transcriptomic: the study of the complete RNA content in individual cells or organisms.

Advances in the development of bioengineered 3D breast cancer models

Amongst the different types of tumors found in the worldwide population, breast cancer is the most common type of malignancy and the second leading cause of death in women [19]. Although about 90% of the patients with localised breast cancer show a greater than 5-year survival rate, in the case of invasive and metastatic disease, the percentage drops to 30%, with patients having unmet clinical needs and requiring effective therapeutic regimens [20]. Treatment efficacy is usually related to the tumor grade and the expression of specific markers. In addition, extrinsic factors [21] and the specifically mutated cell type in the mammary tissue will determine a more local or invasion-prone type of tumor [22]. Traditional breast cancer characterization is usually based on the presence or absence of hormone receptors (namely oestrogen and progesterone) and human epidermal growth factor receptor 2 (HER2). Thanks to recent advances in genomic and histological analysis, this identification has now been expanded upon, revealing up to 19 different breast cancer subtypes [23,24]. Each breast cancer category, and thus each patient, can respond differently to therapy by harbouring intrinsic changes to different compartments of the cell molecular machinery.

To gain a deeper understanding of the molecular changes and their influence on the efficacy of treatments, researchers have implemented the development of different types of advanced 3D breast cancer models [25–27] (Figure 2). Specific types of breast cancer and stromal cells can be used (Figure 2A), combined in different aECMs to support their growth (Figure 2B). The support matrices can undergo different bioengineering processing to mimic the desired native architecture (Figure 2C), depending on the specific mechanisms under investigation. The incorporation of microfluidic devices has led to new models termed 'tumor-on-a-chip' [28,29]. By means of applying principles of fluid dynamics and **microfluidics** technologies, different types of cells, including patient-derived ones, can be cultured with their specific media composition and with very small volume requirements (Figure 2D and [30]). In addition, chips can be

Key figure

Spatial biology for enhanced characterization of breast cancer

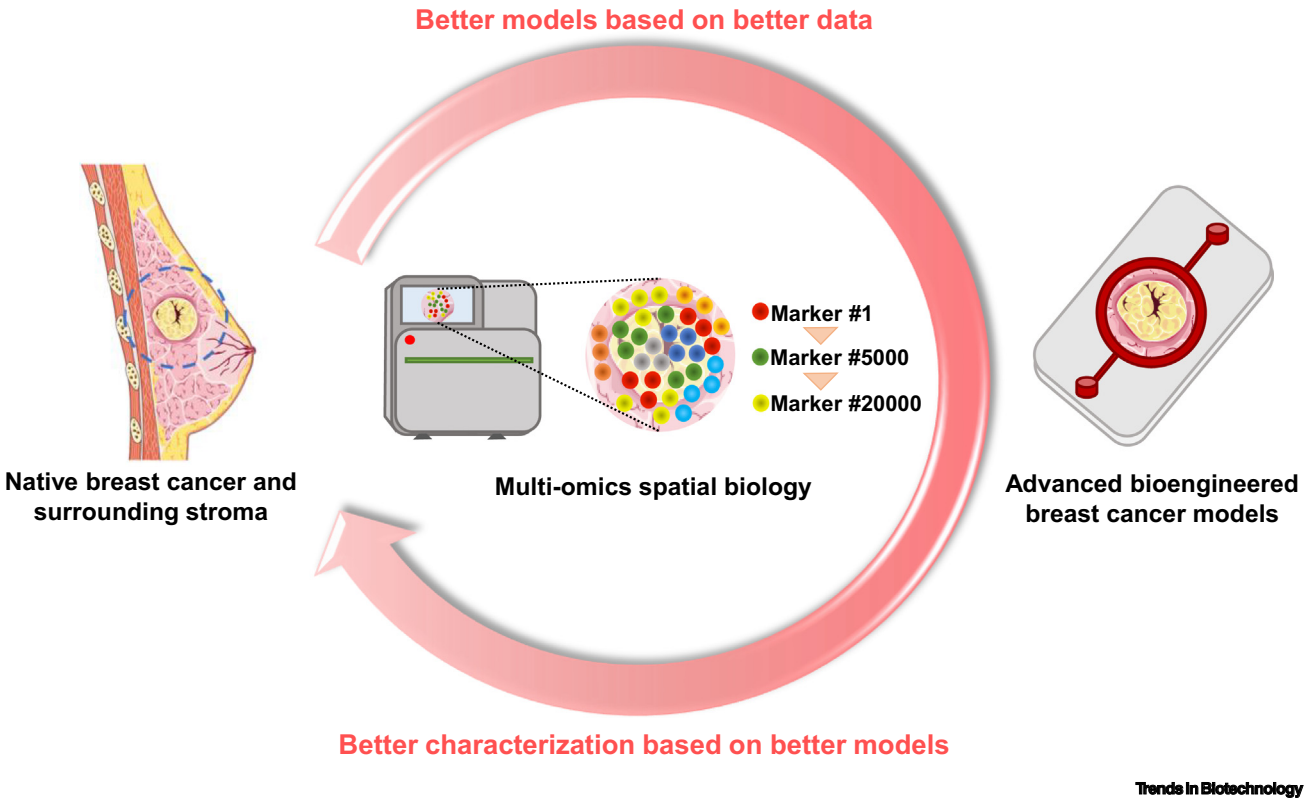

Figure 1. Extensive understanding at the molecular level is necessary to fully comprehend breast oncogenesis. Standard -omics analysis techniques (e.g., genomics, transcriptomics, proteomics) often fail to provide information regarding tissue architecture and cancer cell–stroma interaction. In this regard, breast cancer models can overcome this issue by bioengineering a specific cellular and structural microenvironment, even though their biological characterization frequently lacks complex tissue information. Multiomics spatial biology techniques can help overcome these drawbacks, encompassing both a deeper structural understanding and characterization of the interaction between cancer and stromal cells, at cell resolution, for a specific tumor subtype. The obtained architectural data can improve the development and characterization of the bioengineered models, which in turn can lead to a better understanding of breast cancer development and thus accurate testing of novel treatments.

fabricated with precisely tailored designs towards the tissue architecture of interest to investigate the desired biological mechanism [31–33]. Another technology that has lately gained interest in the 3D tumor models field is bioprinting. Thanks to the possibility of bioengineering the material that will serve as aECM, different bioinks can be produced [34,35]. After the addition of cells, constructs can be bioprinted with specific architecture, different degrees of complexity, and in a reproducible manner [26,36,37] (Figure 2E).

By means of using specifically bioengineered breast cancer models, novel anticancer drugs with clinical potential have been tested [30,38,39]. In this context, spheroids have been widely used as a simplistic 3D model for drug testing applications. For example, Chen *et al.* designed a multichannel microfluidic device to investigate the efficacy of doxorubicin-loaded nanocarriers on breast cancer multicellular spheroids [40]. In this way, researchers were able to monitor in real time the nanocarriers' diffusion and penetrability into the mimicked endothelial, ECM, and

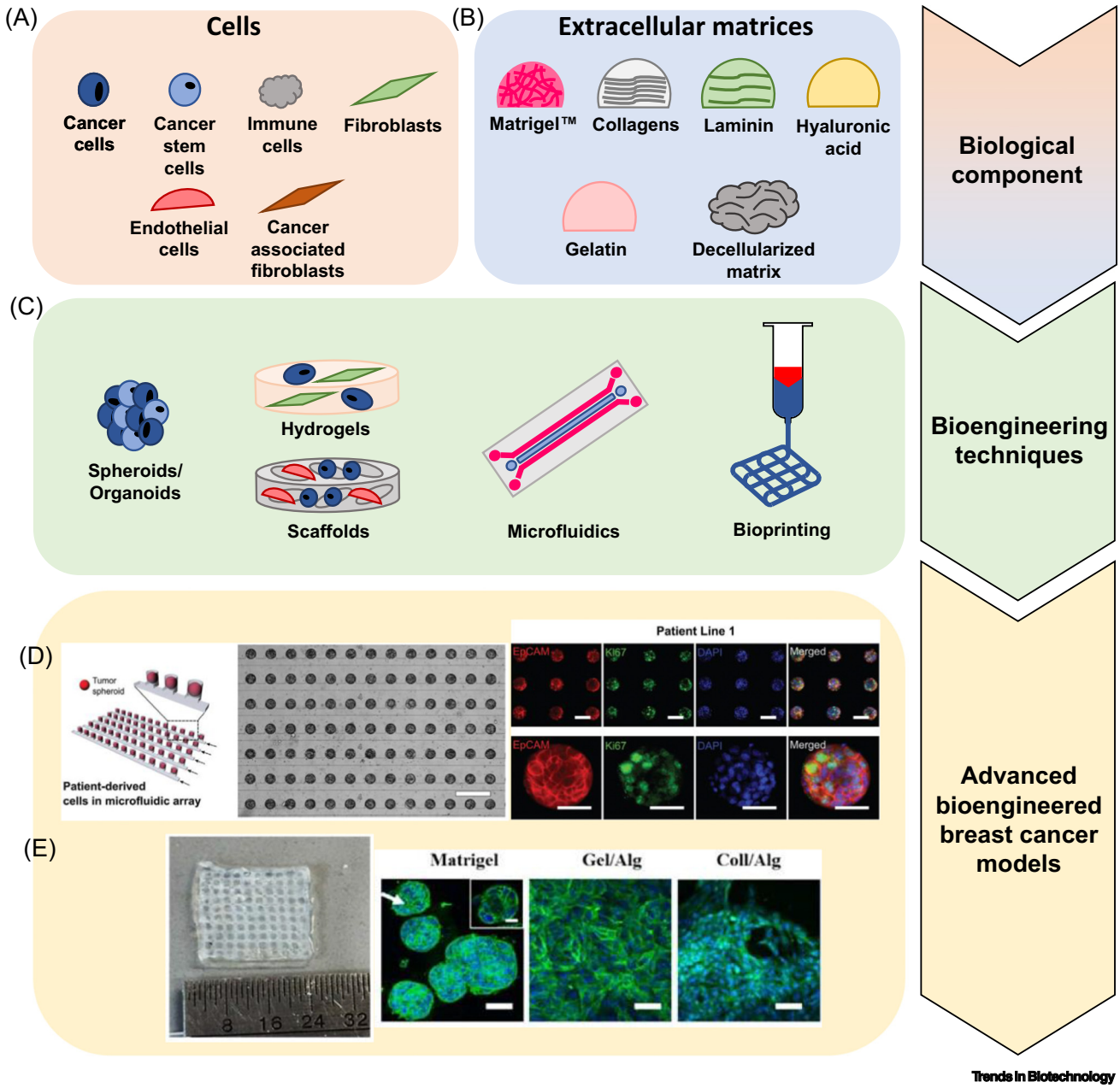

Figure 2. Pipeline for 3D model development. The combination of diverse cell types (A) with an appropriate artificial extracellular matrix support (B) alongside different bioengineering techniques (C) allow the development of 3D breast cancer models with specifically tuned characteristics. Various fabrication methods are available, ranging from organoids and scaffold-based models to more complex and advanced systems, such as microfluidics and bioprinting. (D) Example of a microfluidic array system used to produce patient-derived spheroids to test and compare drug efficacy observed *in vivo* [30]. (E) Example of bioprinted construct with specific design, using Matrigel, gelatin/alginate (Gel/Alg), or collagen/alginate (Coll/Alg) bioinks [26]. In general, the possibility to choose between this multitude of processing techniques provides versatility tailored to the specific application. Images from (D) [30] and (E) [26] are adapted and reproduced with permission.

tumor compartments, thus having potential for fast drug screening applications [40]. Han and colleagues were able to bioprint distinctly both the ductal and tumor components using different breast cancer cells, closely resembling the breast tissue microarchitecture observed in humans

[41]. They also observed a differential drug response relative to the one observed in patients when mimicking an advanced cancer stage. The emergence of organoids compared with spheroid-based 3D models led to a further improvement in the field, with the possibility to better preserve the cellular composition of patients' mammary tissue and its basic architecture [42,43]. Parigoris *et al.* were able to develop self-assembling epithelial mammary organoids with a basal phenotype to study the impact on the invasiveness of metastatic MDA-MB-231 cells [44]. They observed that breast cancer cells follow a specific invasive pattern starting from epithelial cells to the basal side of the basement membrane, and its integrity influences cancer cells' invasiveness [44].

Besides recreating the TME environment, the inclusion of the vascular component is essential for the development of a complete 3D model. In fact, neovessel formation is one of the step markers of cancer progression, promoting not only higher flows of nutrients but also the infiltration and entrance into the main blood circulation of invasive breast cancer cells [45]. On this note, dynamic cues are also important to understand the behaviour of circulating tumor cells and the mechanisms underlying distant tissue site invasion and metastasis formation [46]. In addition, a dynamic flow system can retrieve additional information on metabolites secreted by cancer cells, which could be used for novel drug discovery [46].

Bioengineered 3D breast cancer models still face difficulties in validation in both laboratory and clinical settings

Despite the described important advancements brought to the field, 3D breast cancer models still face many challenges. As previously mentioned, 3D tumor modelling often relies on the use of cancer spheroids or bioengineered matrices tailoring a specific part of the ECM. Although they might be useful for initial screening studies, they are in fact oversimplistic, not taking into consideration the complete set of cellular or matrix components of the TME of interest. Moreover, lack or misrepresentation of vascularization might hinder the obtained biological relevance. It is unlikely that results obtained from avascular bioengineered models can match the observed *in vivo* breast cancer behaviour. Instead, when the blood supply is indeed mimicked, human umbilical vein endothelial cells are the most used because of their relatively easy culture conditions. Nonetheless, they are not always biologically representative of the type of vasculature present in the tumor and stroma tissue bulk, usually being capillaries and microvasculature cells. In addition, to assess the performance of a novel biomaterial matrix to be used for 3D modelling, it is common to use cancer cell lines corresponding to the tumor of interest. Even though their nonstrict media requirements make them easy to culture, cell lines present aberrant metabolic pathways that differ from the breast cancer development in patients, especially if distant metastatic sites are taken into consideration [47]. All the above-mentioned factors can influence the results and assessment of drug testing performed on the 3D models, thus their human-like responses. For this reason, to develop functional devices for drug screening, all the different specific subtypes of breast cancer should be well represented, with models that are clinically validated [48,49]. Comparison of advanced models with relevant clinical specimens is a key validation step that is often overlooked and that can now theoretically be carried out using more advanced endpoint analyses such as spatial -omics. To progress from drug development towards personalised medicine, models will, of course, need to be further tailored to become patient-specific [50]. This depends on their clinical history and the combination of treatments received, which ultimately influence their genomic landscape and thus the drug response [51,52].

By far, the biological evaluation of even complex models has often relied on underpowered endpoint assays. For example, it is common practice to evaluate cell behaviour based on assessment of either small, specific panels of single genes (e.g., via quantitative real-time PCR)

or proteins (by immunocytochemical imaging) [53–55]. Although these analyses are useful for hypothesis-driven research around known phenotypes, they are not informative for discovery research of unknown phenotypes, less commonly studied genes, or the broader picture in general. This undermines the amount and the quality of the biological information obtained and thus their relevance for the mimicry of a specific breast cancer subtype. We previously highlighted the great heterogeneous diversity present in breast tumors following histological classification [56,57]. Ideally, 3D models should be well engineered to recapitulate each one of the 19 and counting different breast cancer subtypes in order to have useful platforms for preclinical research [58]. But this goal cannot be achieved if we do not have precise endpoints that can properly characterise them. Mutai *et al.* observed that a more distinct subdivision of HER2 expression at the histological level, including low and zero levels, can be a prognostic factor for treatment outcomes in early stage oestrogen receptor-positive patients with breast cancer [59]. This strengthens the fact that there is a great need for advanced techniques to assess the specific position in which sets of genes, RNA, and proteins are expressed throughout the cancer tissue to fully gather relevant information on its formation and progression.

Bridging the gap: the advent of spatial multiomics techniques

Detailed genomic, transcriptomic, and proteomic analyses are vital to fully understand breast cancer biology and thus to fully characterise and validate the derived bioengineered 3D breast cancer models [60]. The past two decades have seen the rise of numerous -omics technologies and platforms (Figure 3A) applied either to DNA, RNA, protein, or epigenetic levels. Different techniques are available with a diverse magnitude of data throughput [61,62] (Table 1), depending on the specific extent of biological information needed. Standard -omics analysis for tissue samples includes whole-genome and whole-exome analysis via microarrays and/or direct sequencing [63]. However, these bulk analyses overlook the vast cellular heterogeneity present across the tumor and surrounding stroma in the TME (Figure 3B). In past years, single-cell sequencing has gained popularity and can be used to overcome that limitation. Specifically, RNA sequencing (RNA-seq) of single breast cancer cells can unravel important insights into clonal cell proliferation and the establishment of circulating tumor cells. Padmanaban *et al.*, for example, demonstrated the dual role of E-cadherin expression in different types of invasive breast cancer when initiating dissemination and metastatic seeding [64]. The in-depth information obtained with single-cell techniques is impressive, but these techniques fail to provide details regarding the specific spatial localization within the highly heterogeneous breast cancer TME architecture (Figure 3B).

Thus, novel approaches that molecularly characterise and account for the precise spatial localization of different cell types within the TME are vital to fully unravel breast cancer biology. In the past couple of years, a plethora of spatially resolved -omics techniques have been undergoing development to try to tackle this issue in the cancer research field. One of the first to thrive and gain great interest was spatial transcriptomics [65]. Several companies offer different methodologies, with leading technological platforms being Visium (from 10x Genomics)[i] and GeoMx Digital Spatial Profilers (from NanoString)[ii]. In general, these types of spatial transcriptomic analyses rely on multiple barcoded probes, each corresponding to a specific transcript. They can either be immobilised on a glass support or hybridised onto the breast tissue section of interest (Figure 3C). After binding to the histological section, precise mapping and localization of the obtained differential transcript levels is done through imaging and bioinformatic analysis (Figure 3C). Hence, this methodology allows the investigation of differentially expressed genes in different cell types spread across the tumor and stroma. This can be particularly useful for highly histologically heterogeneous cancers such as breast cancer. Advances to reach single-cell resolution have been made in the new upcoming technological platforms, including Visium HD,

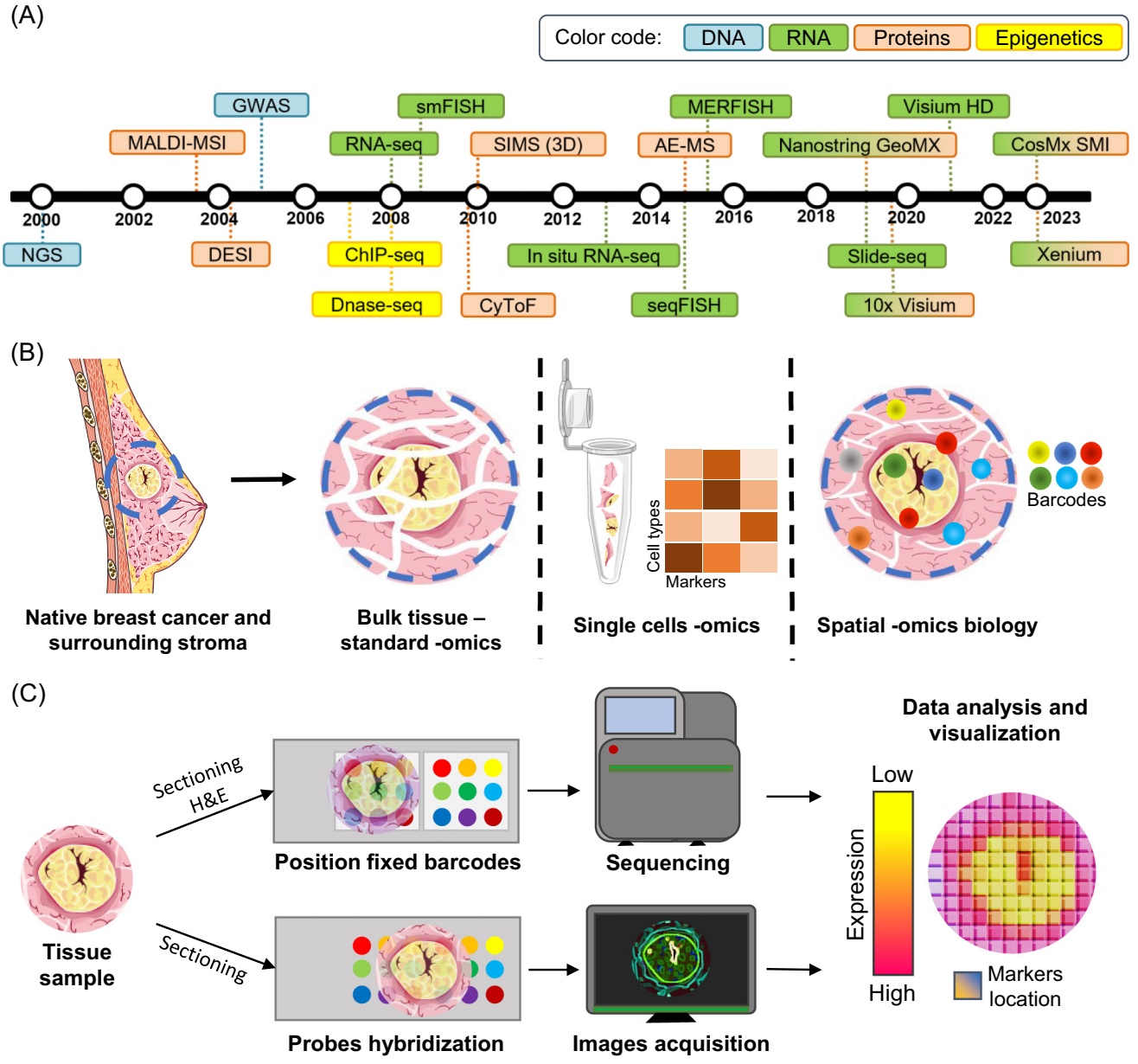

Figure 3. Advances in spatial -omics technologies for 3D breast cancer model research. (A) Timeline summarising the advent of different -omics platforms over time. The technologies are represented with different colors regarding whether they are applied to DNA (blue), RNA (green), proteins (orange), or chromatin (yellow). Mixed colors represent techniques that can be applied to different categories. (B) Progress and differences between different -omics techniques. Standard bulk tissue analysis can provide general information about breast cancer and stroma interaction, but not about specific cell–cell interaction. On the contrary, single-cell -omics can overcome this but does not yield a precise location in the analysed sample. Spatial -omics technologies can do both. (C) Schematics describing the general workflow for spatial transcriptomic analysis. The tissue of interest is sectioned and histologically stained. Sections are then bound to different barcoded probes corresponding to specific transcripts. The barcodes can be either immobilised on a glass support or hybridised onto the histological section. By employing imaging and bioinformatic tools, the precise localization of the differentially expressed transcripts is obtained. Abbreviations: AE-MS, affinity enrichment mass spectrometry; ChIP-seq, chromatin immunoprecipitation sequencing; CosMx SMI, CosMx spatial molecular imager; CyToF, mass cytometry; DESI, desorption electrospray ionization; Dnase-seq, DNase sequencing; GWAS, genome-wide association study; H&E, haematoxylin and eosin; MALDI, matrix assisted laser desorption/ionization; MERFISH, multiplexed error robust fluorescence *in situ* hybridization; MSI, mass spectrometry imaging; NGS, next-generation sequencing; RNA-seq, RNA sequencing; seqFISH, sequential fluorescence *in situ* hybridization; SIMS 3D, secondary ion mass spectrometry 3D imaging; Slide-seq, slide sequencing; smFISH, single-molecule fluorescence in situ hybridization.

Table 1. Technological platforms available for standard and spatial -omics

Technique	Full name	Analyte investigated				Type of analysis	Spatial biology information?
		DNA	RNA	Proteins	Epigenetics		
NGS	Next-generation sequencing	x	–[a]	–	–	Bulk, large-scale DNA sequencing of the entire genome or whole exome [76]	No
GWAS	Genome-wide association study	x	–	–	–	Large-scale genome sequencing of large numbers of subjects to find genetic variants correlated with a specific disease [76]	No
ChIP-seq	Chromatin immunoprecipitation sequencing	–	–	–	x	Combination of ChIP with NGS to profile genome-wide epigenetic patterns [77]	No
DNase-seq	DNase sequencing	–	–	–	x	Genome-wide sequencing of DNase I cleavage regions to identify the location of regulatory proteins [77]	No
RNA-seq	RNA sequencing	–	x	–	–	Gene expression, large-scale sequencing of the entire transcriptome, including RNA coding and noncoding regions [78]	No
smFISH	Single-molecule fluorescence *in situ* hybridization	–	x	–	–	Single-cell gene expression and subcellular localization of specific individual RNA molecules [79]	Yes, but only for a specific RNA molecule
In situ RNA-seq	*In situ* RNA sequencing	–	x	–	–	Gene expression data for different markers at subcellular resolution on fixed tissue samples [79]	Yes, but only for a small number of genes
seqFISH	Sequential fluorescence *in situ* hybridization	–	x	–	–	*In situ* single-cell gene expression profile, using different hybridising fluorescent probes [79]	Yes, but only single-cell resolution
MERFISH	Multiplexed error robust fluorescence *in situ* hybridization	–	x	–	–	Single-cell, simultaneous measurement of hundreds to thousands of RNA transcripts, preserving spatial distribution [79]	Yes, but only single-cell resolution
Slide-seq	Slide sequencing	–	x	–	–	Broad RNA sequencing of gene expression in complex tissue sections, using glass surfaces covered with DNA-barcoded beads having known positions, at 10-μm resolution [79]	Yes
NanoString GeoMx	–	–	x	x	–	Spatial transcriptomic and proteomic analysis of defined regions of interest in tissue sections using glass slides with immobilised barcoded probes [80]	Yes
10x Visium	–	–	x	x	–	Spatial transcriptomic and proteomic analysis of whole-tissue sections using glass slides with immobilised barcoded probes [80]	Yes
Visium HD	–	–	x	–	–	Spatial transcriptomic analysis of whole-tissue sections, with single-cell resolution (not commercialised yet)	Yes
Xenium	–	–	x	x	–	High-plex, *in situ*, spatial multiomics platform (transcriptomics and proteomics) for tissue samples at subcellular/single-cell resolution [80]	Yes
CosMx SMI	CosMx spatial molecular imager	–	x	x	–	High-plex, *in situ*, spatial multiomics platform (transcriptomics and proteomics) for tissue samples at subcellular/single-cell resolution [80]	Yes
MSI	Mass spectrometry imaging	–	–	x	–	Proteomic analysis to identify and quantify metabolites and proteins in a sample, ranging between small molecules, peptides, glycans, lipids, and protein complexes [81]	Yes

(continued on next page)

Table 1. (continued)

Technique	Full name	Analyte investigated				Type of analysis	Spatial biology information?
		DNA	RNA	Proteins	Epigenetics		
AE-MS	Affinity enrichment mass spectrometry	–	–	x	–	Proteomic analysis to study protein–protein interaction [82]	No

[a]Not applicable.

Xenium (10x Genomics), and CosMx (NanoString). Some of these technologies can also be applied to proteomics, allowing spatial multiomics analysis of specimens. GeoMx, for example, offers a panel of more than 96 proteins. Another emerging company in the field, Akoya[iii], offers highly multiplexed, ready-to-use key biomarker panels involved in tumor and TME interaction, facilitating spatial biology analysis.

Implementation of these approaches and combination with other advanced analytical techniques is essential to fully study the spatial biology of breast cancer. For example, determining the localization of specific molecules and metabolites across the TME could unravel novel possible targets for treatment or guide treatment regimens to a specific patient. Recent advancements in mass spectrometry imaging (MSI) have paved the way for mapping specific analytes across a sample.

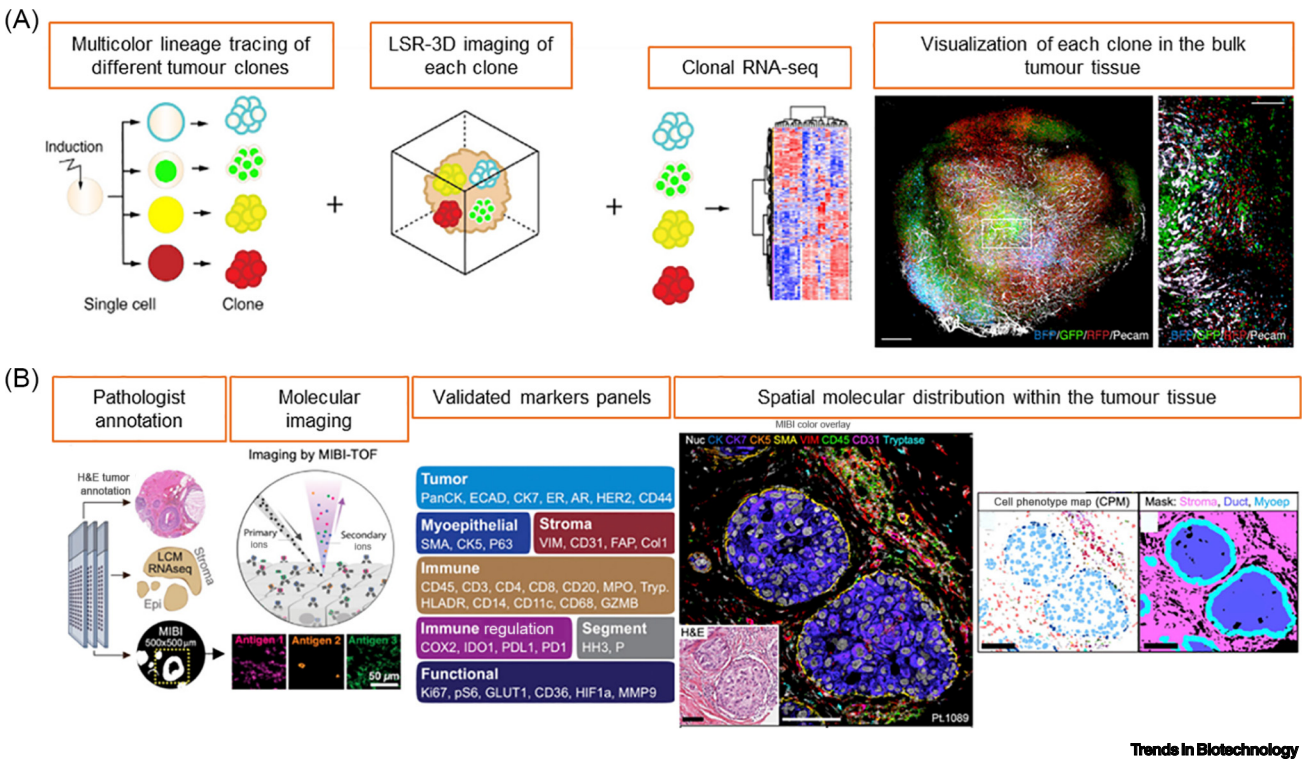

Figure 4. Examples of spatial -omics technology applications. (A) Large-scale single-cell resolution 3D (LSR-3D) imaging of the clonal lineage of different breast cancer cell subpopulations, with subsequent localization in the tumor tissue [61]. Coupled RNA sequencing (RNA-seq) analysis identified the gene expression profile of each specific clone. (B) Multiplexed ion beam imaging by time of flight (MIBI-ToF) employed to study the tumor microenvironment (TME) molecular changes underlying the invasiveness of ductal carcinoma *in situ* (DCIS) [74]. Patient-coupled histological sections derived from DCIS and invasive regions underwent MIBI-ToF imaging using different fluorescently labelled markers to track their spatial distribution within the TME and tumor tissue. An example of a MIBI-ToF image reconstruction is shown. Images in (A) [61] and (B) [74] were adapted and reproduced with permission. Abbreviation: LCM, laser capture microdissection.

Various techniques are available, depending on the different compounds under analysis [66], ranging from small molecules to peptides, glycans, lipids, and protein complexes. Matrix-assisted laser desorption/ionization (MALDI) and secondary ion mass spectrometry (SIMS) are two of the most commonly used MSI techniques [67]. MALDI uses a laser beam to scan the sample of interest covered with a photoactive matrix, and it is used mainly to identify proteins, metabolites, and lipids. SIMS instead uses ion beams as a probe and does not require a photomatrix. This technique can help to identify ions, small molecules, and protein fragments [67]. Another MSI method frequently used is desorption electrospray ionization (DESI) [68]. As compared with MALDI and SIMS, DESI does not require sample modifications. Thus, it retains intact proteins' post-translational modifications, making it possible to identify different isoforms within a specific cell type [69]. Reaching single-cell resolution in some cases, MSI can provide additional information about the structure and chemical composition of a specific breast cancer tissue sample [70] and can complement spatially resolved transcriptomic data. In particular, at single-cell level, mass cytometry analysis can implement insights regarding the immune landscape in the breast tissue and sample of interest [71], which is of great importance in contexts of immune evasion. This poses a great advantage for exploring mechanisms of breast cancer cell interaction with the TME compartment [72]. A key advancement from Wu *et al.* showcases a human breast cancer atlas with spatially resolved tissue architecture details, which identified different clinically relevant clusters [73]. Rios *et al.* recently developed a novel method to optically clear, label, and 3D image breast tumors at high resolution with single-cell resolution (Figure 4A) [61]. By fluorescently labelling diverse cell populations and RNA-seq analyses, the researchers were able to track each cell clone's expression profile and specific position in the tumor mass [61]. Risom and colleagues indeed demonstrated the importance of specific TME localization of fibroblasts and immune and myoepithelial cells when ductal carcinoma *in situ* and invasive breast cancer were compared (Figure 4B) [74]. In general, the outcome is a deeper understanding of breast cancer tissue architecture and biology, which is needed to engineer more biomimetic breast cancer models [75]. To complete the cycle (as outlined in Figure 1), spatial -omics analysis should be carried out on future advanced breast models in order to compare with clinical samples and confirm biomimicry to a degree not yet shown.

Outstanding questions

How can we guarantee that we effectively instruct cancer cells in models with the appropriate biological and biomechanical cues found in the surrounding tumor microenvironment? For this purpose, are more suitable biomaterials used as artificial extracellular matrices required?

How can we ensure that the developed bioengineered 3D breast cancer models accurately reflect the specific tumor subtype for being clinically relevant in personalised medicine? Small differences in the genomic, transcriptomic, proteomic, metabolomic, and epigenomic landscape will change each patient's response to a specific combination treatment locally, although in the literature most identified alterations are presented at the bulk sequencing level.

How can the latest advancements in high-throughput technologies, in particular spatial biology, help to boost the validation and development of bioengineered 3D breast cancer models and thus bridge the gap between laboratory research and clinical application?

Concluding remarks and future perspectives

As a more ethical and biologically relevant alternative to animal models, complex 3D *in vitro* breast cancer models are being developed. Precise bioengineering of the surrounding stroma allows a more controlled and appropriate behaviour of breast cancer cells, as observed in patients. Although these models have proved useful for fast drug testing and screening, they still face many challenges in validation and translation into the clinic (see Outstanding questions). Multiple subtypes of breast cancer exist, each bearing specific sets of alterations, which ultimately influence the therapy response in each. 3D breast cancer models should accurately reflect the diverse clinical phenotypes observed in human patients to match their landscape. Thus, using patient-derived tumor and ECM cells is a necessary step to fully validate the developed model and to translate its relevance to the clinical setting. From a bioengineering point of view, the use of biomaterials as aECMs and processing techniques is still not fully exploited and deserves a great deal of attention. Moreover, we strongly advocate the inclusion of more in-depth target analyses when developing complex architecturally organised 3D models. Currently, the research field underuses them by not making use of emerging spatial multiomics technologies. Realistically, the tissue-engineered 3D breast tumor models developed so far might harbour additional key findings, which have not actually been discovered due to a lack of in-depth spatial analysis. The latest high-throughput spatial -omics analyses can provide enough detailed information on cellular behaviour, and thus relevance, of the developed system. Extensive understanding of the interaction between breast cancer cells and the TME and the immune landscape is crucial to gain fundamental knowledge on the mechanistic effects that lead to breast cancer

progression, invasion, and metastasis. One aspect that needs to be noted is that not all the types of native tissue or 3D constructs are suitable for spatial -omics. For example, too thick and dense samples would impair the analysis. To overcome this, the use of tissue-clearing agents can be considered, which would lead to more optically clear images for analysis. Additionally, spatial multiomics cannot currently be carried out longitudinally, because all methods are partially or fully destructive of the tissue, making it challenging to assess changes in expression over time. Currently, this can be overcome only by using separate specimens at multiple time points, which adds to the already high cost of running such techniques.

Furthermore, the comparison of spatially resolved -omics datasets between bioengineered 3D breast cancer models and matched human breast cancer specimens will greatly improve their applicability. Further technological platform advancements will pave the way also for spatial epigenomic, metabolomic, and lipidomic analyses, which will be of great value for research to verify in depth the responses of these models to therapeutic agents. However, implementation of -omics technologies in daily research activities can be impaired by the unavailability of equipment in research facilities, the high cost of each specific library preparation and sequencing run, and the highly demanding bioinformatic data analysis. Particularly, computing power and advanced computational tools to analyse the acquired data still represent a bottleneck in these technologies. Collaboration efforts of research centres with different backgrounds and expertise should be highly encouraged to overcome difficult access to spatial technologies. This would greatly improve the efficiency and likelihood of success between bioengineered 3D breast cancer models and full validation for potential clinical settings. In addition, making fully publicly available the multitude of spatial biology generated data, together with well-annotated analysis pipelines, would be very helpful – in essence building on the success of cBioportal[iv] and applying it to the spatial era. Ultimately, this would serve as a platform for researchers from various specialities to consult and derive new valuable information, with additional benefit possible for patients with cancer by making the most of existing data.

In summary, the most exciting advancements developed by bioengineers and molecular biologists must be brought together to overcome the gaps between these fields, so that we can improve bioengineered 3D breast cancer model development, impacting preclinical research and how patients will ultimately receive optimum personalised treatment.

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Declaration of interests

None are declared by the authors.

Resources

[i] www.10xgenomics.com

[ii] www.nanostring.com

[iii] www.akoyabio.com

[iv] www.cbioportal.org

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