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

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Abstract

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 three-dimensional (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 personalized medicine approaches.

Three-dimensional (3D) models as novel tools for cancer research

Historically, laboratory-based cancer research and drug development has employed the use of individual cancer cell lines in two-dimensional (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 tumour cells grow, invade, and metastasise to distant sites [3,4]. To better mimic the physiological cancer tissue microenvironment, the use of extracellular matrices-based 3D culture methods and related development of 3D tumour models has 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 extracellular matrices (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 tumour 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* tumour models development, which will be 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 tumour microenvironment (TME), they still face some challenges and limitations. Standard endpoint analyses such as imaging can only provide a general understanding of the structural organisation 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 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 understand 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 **multi-omics** technologies is now making this possible, allowing the precise identification in the region of interest of a specific marker, at **genomic**, **transcriptomic**, **proteomic** level and beyond. With the emergence of spatial multi-omics 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 cells organisation will allow the continued development of yet more relevant 3D models, and ultimately, efficient anti-cancer 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 personalized 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 multi-omics 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, which 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.

Advances in the development of bioengineered 3D breast cancer models

Amongst the different types of tumours 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 localized breast cancer show greater than 5-years 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 tumour 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 invasive-prone type of tumour [22]. Traditional breast cancer characterization is usually based on the presence or absence of hormone receptors (namely estrogen 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 patient, can respond differently to therapy, by harbouring intrinsic changes to different compartments of the cell molecular machinery.

To gain a deeper understanding on 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 'tumour-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, [30]). In addition, chips can be 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 tumour 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 multi-channel 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 tumour compartments, thus having potential for fast drug screening applications [40]. Han and colleagues were able to bioprint distinctly both the ductal and the tumour component using different breast cancer cells, closely resembling the breast tissue microarchitecture observed in humans [41]. They also observed a differential drug response, as the one observed in patients, when mimicking an advanced cancer stage. The emergence of organoids, as compared to spheroid-based 3D models, lead 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, neo-vessel formation is one of the step marks of cancer

progression, promoting not only higher flows of nutrients, but also the infiltration and entrance in the main blood circulation of invasive breast cancer cells [45]. On this note, dynamic cues are also important to understand the behaviour of circulating tumour cells, and the mechanisms underlying distant tissue sites 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, both in 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 tumour modelling often relies on the use of cancer spheroids or bioengineered matrices tailoring a specific part of the ECM. Though 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 (HUVEC) are the most used, due to their relatively easy culture conditions. Nonetheless, they are not always biologically representative of the type of vasculature present in the tumour 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 tumour of interest. Even though their non-strict 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 which is often overlooked, and which can now theoretically be carried out using more advanced endpoint analyses like spatial omics. To progress from drug development towards personalized 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 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 immunocytochemistry imaging) [53–55]. While 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 for 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 tumours following histological classification [56,57]. Ideally, 3D models should be well engineered to recapitulate each single one of the 19 and counting different breast cancer subtypes, in order to have useful platforms for preclinical research [58]. But this goal is not possible to 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 level, can be a prognostic factor for treatment outcomes in early stage ER+ breast cancer patients [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 multi-omics techniques

Detailed genomic, transcriptomic, and proteomic analyses are vital to fully understand breast cancer biology, and thus to fully characterize and validate the derived bioengineered 3D breast cancer models [60]. The last 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 diverse magnitude of data throughput [61,62] (Table 1), depending on the specific extent of biological information needed. Standard -omics analysis for tissue samples include 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 tumour 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 tumour 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 they fail to provide

details regarding the specific spatial localization within the highly heterogeneous breast cancer TME architecture (Figure 3b).

Thus, novel approaches that molecularly characterize and account for the precise spatial localization of different cell types within the TME are vital to fully unravel breast cancer biology. In the last 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 transcriptomics analyses rely on multiple barcoded probes, each corresponding to a specific transcript. They can either be immobilized on a glass support or hybridized onto the breast tissue section of interest (Figure 3c). After binding to the histological section, precise mapping and localization of the obtained differential transcripts levels is done through imaging and bioinformatics analysis (Figure 3c). Hence, this methodology allows the investigation of differentially expressed genes in different cell types spread across the tumour and stroma. This can be particularly useful for highly histologically heterogeneous cancers such as breast. Advances to reach single cell resolution have been made in the new upcoming technological platforms, including Visium HD, Xenium (10x Genomics) and CosMx (NanoString). Some of these technologies can also be applied to proteomics, allowing spatial multi-omics analysis of specimens. GeoMx for example offer a panel of more than 96 proteins. Another emerging company in the field, named Akoya[iii], offer highly multiplexed, ready-to-use key biomarkers panels involved in tumour 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. Various techniques are available depending on the different compounds under analysis [66], ranging from small molecules, peptides, glycans, lipids and protein complexes. Matrix-Assisted Laser Desorption/Ionization (MALDI) and Secondary Ion Mass Spectrometry (SIMS) are some 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 photo-matrix. 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 complement spatially-resolved transcriptomic data. In particular, at single-cell level, mass cytometry (CyToF®) 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 cells 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]. The group of Rios et al. recently developed a novel method to optically clear, label and 3D-image breast tumours 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 tumour mass [61]. Risom and colleagues indeed demonstrated the importance of specific TME localization of fibroblasts, immune and myoepithelial cells, when ductal carcinoma *in situ* and invasive breast cancer are 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 back to clinical samples and confirm biomimicry to a degree not yet shown.

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 proven 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 tumour 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 artificial extracellular matrices 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 underutilises them by not making use of emerging spatial multi-omics technologies. Realistically, the tissue engineered 3D breast tumour 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 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 multi-omics cannot currently be carried out longitudinally, as all methods are partially or fully destructive of the tissue, making it challenging to assess changes in expression over time. Currently this can only be overcome 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 epigenomics, metabolomics and lipidomics 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-based 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 cancer patients 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 personalized treatment.

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Declaration of interest

None are declared by the authors.

Resources

[i] www.10xgenomics.com/

[ii] www.nanostring.com/

[iii] https://www.akoyabio.com/

[iv] www.cbioportal.org/

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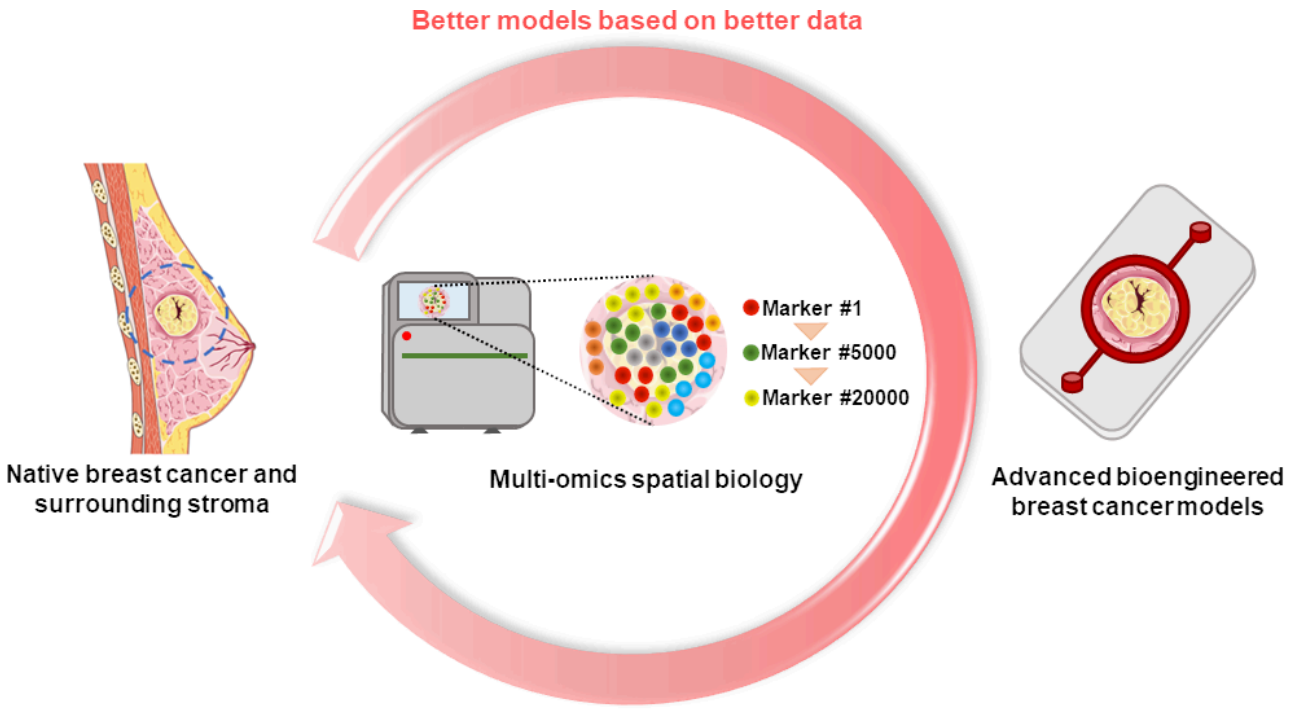

Figure 1. **Key figure. Spatial biology for enhanced characterization of breast cancer**. Extensive understanding at the molecular level is necessary to fully comprehend breast oncogenesis. Standard -omics analysis techniques (e.g., genomics, transcriptomics, proteomics) often fail to provide information 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. Multi-omics 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 tumour 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, thus accurate testing of novel treatments.

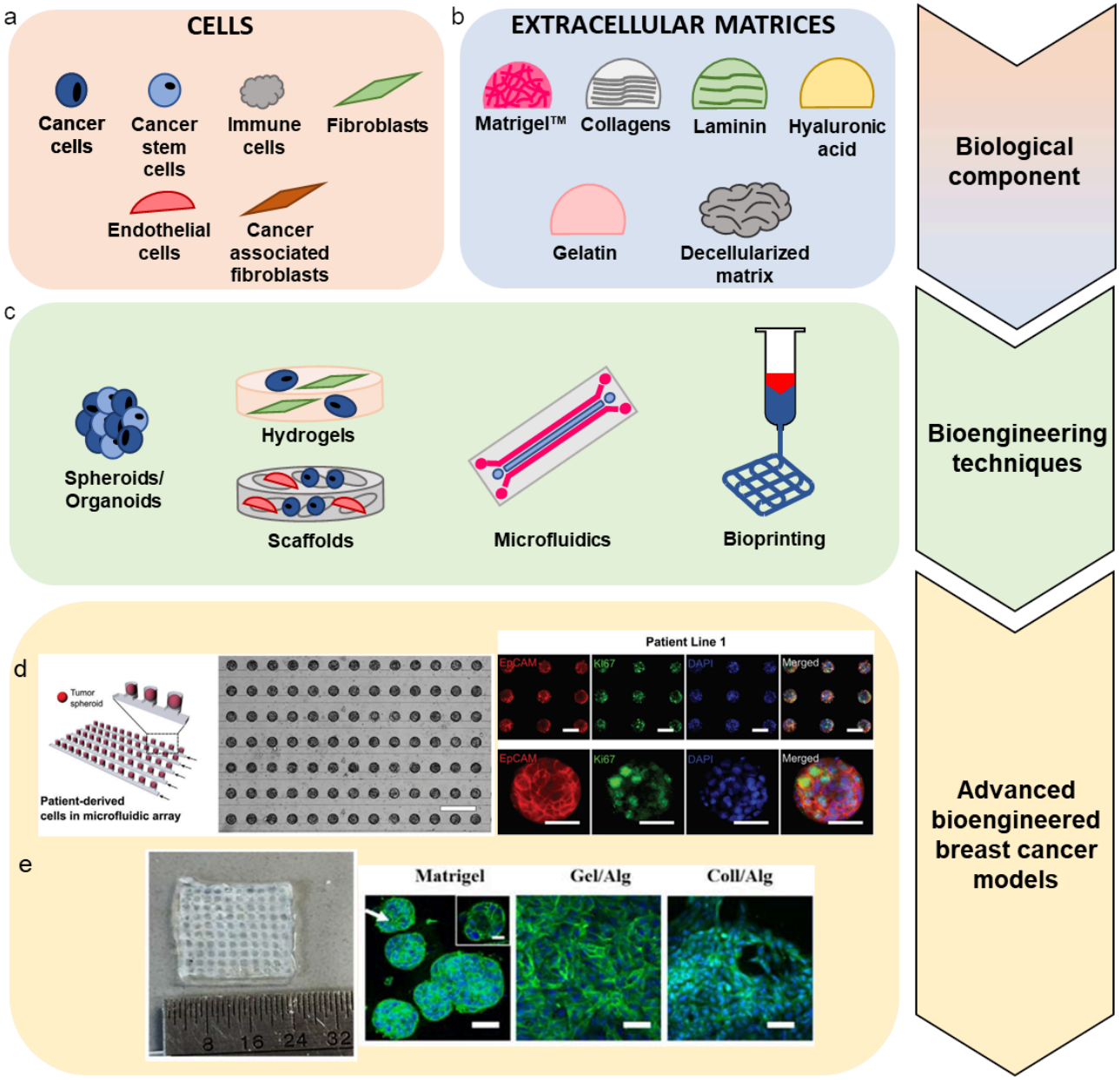

Figure 2. Pipeline for 3D models development. The combination of diverse cell types (a) with an appropriate aECM support (b), alongside different bioengineering techniques (c), allow the development of 3D breast cancer models with specifically tuned characteristics. Various fabrication methods are available, 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's 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.

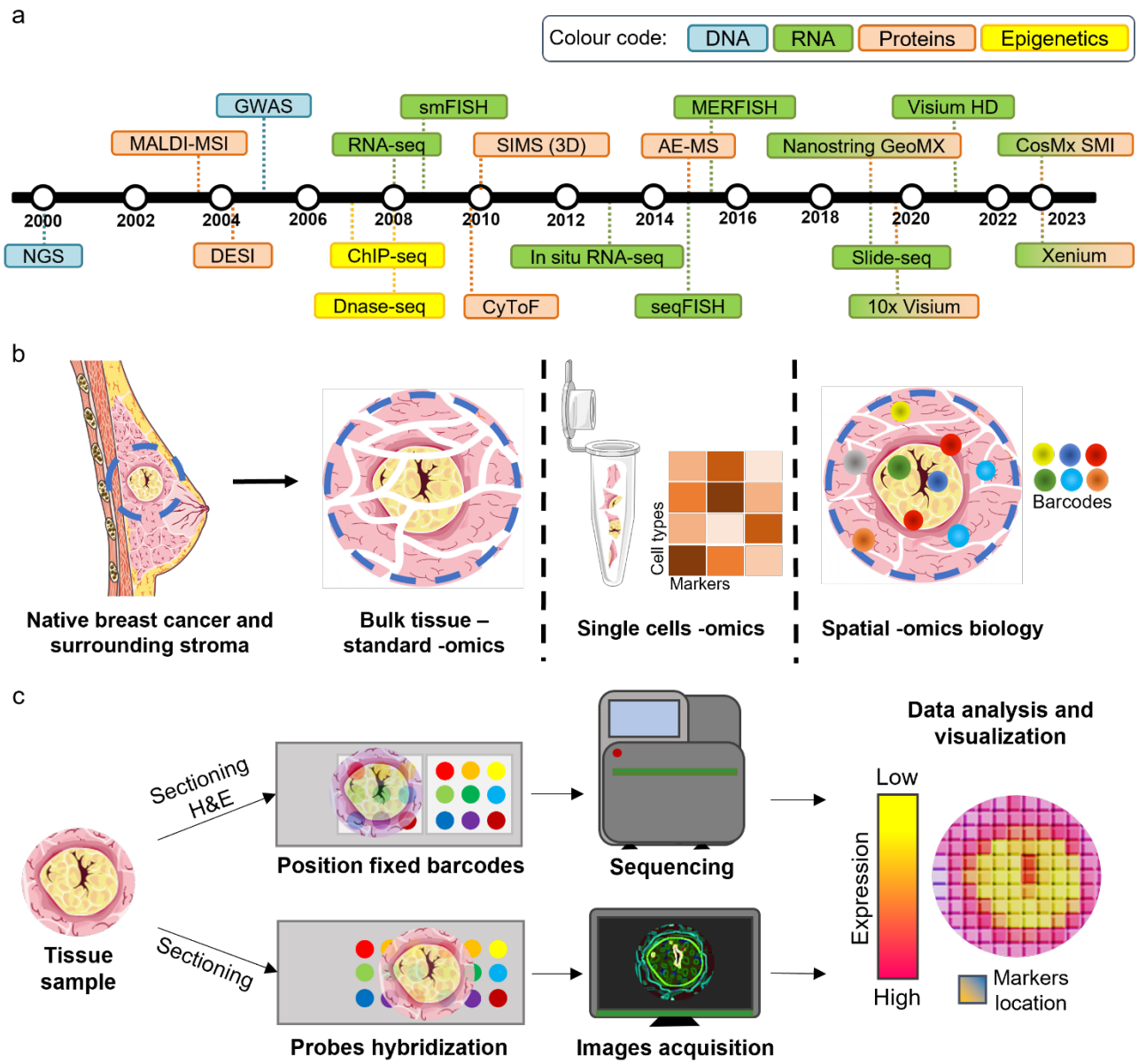

Figure 3. Advances in spatial -omics technologies for 3D breast cancer models research. (a) Timeline summarizing different -omics platforms advent over time. The technologies are represented with different colours whether they are applied to DNA (blue), RNA (green), proteins (orange) and chromatin (yellow). Mixed colours 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 transcriptomics 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 either be immobilized on a glass support or hybridized 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), In situ RNA-seq (In situ RNA sequencing), MALDI-MSI (Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging), MERFISH (Multiplexed Error Robust Fluorescence In Situ Hybridization), NanoString GeoMX, NGS, 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), Visium HD, 10x Visium, Xenium.

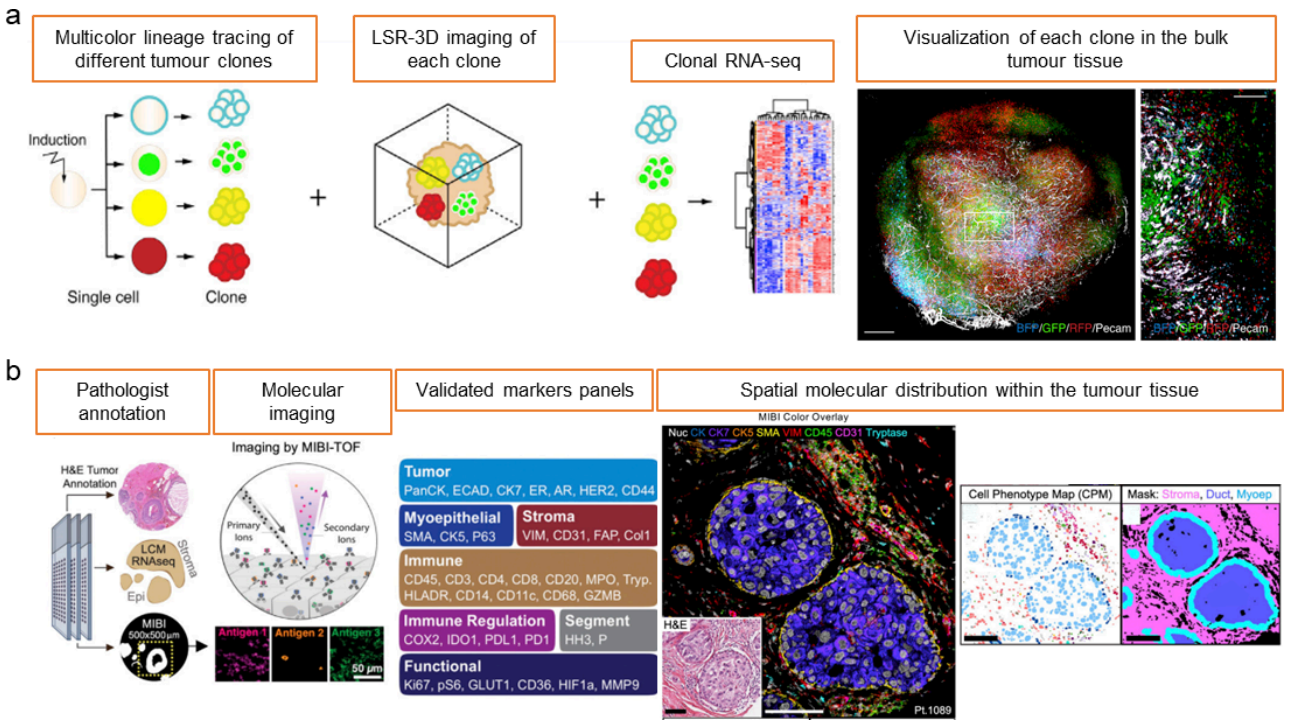

Figure 4. Examples of spatial -omics technology applications. (a) Large-scale Single-cell Resolution 3D (LSR-3D) imaging of the clonal lineage of different breast cancer cells subpopulations, with subsequent localization in the tumour tissue [61]. Coupled RNA-sequencing (RNA-seq) analysis identified the gene expression profile of each specific clone. (b) Multiplexed Ion Beam Imaging by Time of Flight (MIBI-ToF) employed to study the TME molecular changes underlying 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 tumour tissue. An example of a MIBI-ToF image reconstruction is shown. Images in a [61] and b [74] were adapted and reproduced with permission.

Glossary

Biomaterials: natural or synthetic substances designed to interact with biological systems.

Bioprinting: the use of three-dimensional printing technology with materials that incorporate viable living cells.

Genomic: the study of the complete DNA sequence of organisms.

Matrigel™: a commercially available solubilized basement membrane matrix secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells.

Microfluidics: the science of manipulating and controlling fluids, usually in the range of microliters (10^{-6}) to picoliters (10^{-12}), in networks of channels with dimensions from tens to hundreds of micrometers.

Multi-omics: the simultaneous measurement and combination of two or more -omics data sets 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.

Table 1. List of 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	–	–	–	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 hybridizing fluorescent probes [79]	Yes, but only single-cell resolution
MERFISH		–	x	–	–		Yes,

	Multiplexed Error Robust Fluorescence *In Situ* Hybridization					Single-cell, simultaneous measurement of hundreds to thousands of RNA transcripts, preserving spatial distribution [79]	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	–[a]	–	x	x	–	Spatial transcriptomic and proteomic analysis of defined regions of interest in tissue sections, using glass slides with immobilized barcoded probes [80]	Yes
10x Visium	–	–	x	x	–	Spatial transcriptomic and proteomic analysis of whole tissue sections, using glass slides with immobilized barcoded probes [80]	Yes
Visium HD	–	–	x	–	–	Spatial transcriptomic analysis of whole tissue sections, with single-cell resolution (not commercialized yet)	Yes
Xenium	–	–	x	x	–	High-plex, *in situ*, spatial multi-omics 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 multi-omics platform (transcriptomics and proteomics) for tissue samples, at subcellular/single-cell resolution [80]	Yes
MSI	Mass Spectrometry Imaging	–	–	x	–	Proteomics analysis to identify and quantify metabolites and proteins in a sample, ranging between small molecules, peptides, glycans, lipids and protein complexes [81]	Yes
AE-MS	Affinity Enrichment Mass Spectrometry	–	–	x	–	Proteomics analysis to study protein-protein interaction [82]	No

[a]Not applicable