REVIEW ARTICLE

The biomimetic extracellular matrix: a therapeutic tool for breast cancer research

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Background: A deeper knowledge of the functional versatility and dynamic nature of the ECM has improved the understanding of cancer biology.
Translational Significance: This work provides an in-depth view of the importance of the ECM to develop more mimetic breast cancer models, which aim to recreate the components and architecture of tumor microenvironment. Special focus is placed on decellularized matrices derived from tissue and cell culture, both in procurement and applications, as they have achieved great success in cancer research and pharmaceutical sector.
The extracellular matrix (ECM) is increasingly recognized as a master regulator of cell behavior and response to breast cancer (BC) treatment. During BC progression, the mammary gland ECM is remodeled and altered in the composition and organization. Accumulated evidence suggests that changes in the composition and mechanics of ECM, orchestrated by tumor-stromal interactions along with ECM remodeling enzymes, are actively involved in BC progression and metastasis. Understanding how specific ECM components modulate the tumorigenic process has led to an increased interest in the development of biomaterial-based biomimetic ECM models to recapitulate key tumor characteristics. The decellularized ECMs (dECMs) have emerged as a promising *in vitro* 3D tumor model, whose recent advances in the processing and application could become the biomaterial by excellence for BC research and the pharmaceutical industry. This review offers a detailed view of the contribution of ECM in BC progression, and highlights the application of dECM-based biomaterials as promising personalized tumor models that more accurately mimic the tumorigenic mechanisms of BC and the response to treatment. This will allow the design of targeted therapeutic approaches adapted to the specific characteristics of each tumor that will have a great impact on the precision medicine applied to BC patients. (Translational Research 2022; 247:117–136)

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Abbreviations: BC = Breast Cancer; dECMs = Decellularized Extracellular Matrix; TME = Tumor Microenvironment; CSC = Cancer Stem Cells; Col I = Collagen type I; FN = Fibronectin; EMT = Epithelial-Mesenchymal Transition; TGF-β = Transforming growth factor-beta; BCCs = Breast Cancer cells; LM = Laminin; PG = Proteoglycans; GAG = Glycosaminoglycan; HASPc = Heparan sultafe Proteoglycans; BCSC = Breast cancer stem cells; SDC2 = Syndecan-2; SDC1 = Sydencan-1; GPC = Glypican; Wnt = Wingless; PI3K = Phosphoinositide 3-kinase; HA = Hyaluronan; SLPR = Small Leucine-rich proteoglycan; ERK = Extracellular signal-regulated kinases; DCIS = Ductal carcinoma in situ; VEGF = Endothelial growth factor; TNC = Tenascin; SSP1 = Phosphoprotein 1; THBS = Thrombospondine; POSTN = Periostine; SPARC = Secreted Protein Acidic and Rich in Cysteine; TACS = Tumor Associated Collagen Signatures; CAFs = Cancer-associated fibroblasts; MSCs = Mesenchymal stem cells; TAMs = Tumor-associated macrophages; MMPs = Matrix metalloproteinase; EGFR = Epidermal growth factor receptor; LOX = Lysyl Oxidase; FAP = Fibroblast activation protein; Upa = Urokinase plasminogen; SC = Stem Cell; rBM = Reconstituted basement membrane; EGF = Epidermal Growth Factor; PDGF = Platelet-derived growth factor; DOX = Doxorubicin; MDR1 = Multidrug resistance protein; GelMA = Methacrylate gelatin; PEG = Polyethylene glycol; PEG MAL = Polyethylene glycol maleimide; PEG-PC = Polyethylene glycol phosphocholine; PAM = Polyacrylamide; PLA = Poly-lactic acid; PGA = Poly-glycolic acid; PLGA = Poly lactic-co-glycolic acid; PTX = Paclitaxel; ECSs = Embryonic stem cells; SDS = Sodium deoxysulfate; SLES = Sodium lauryl Ether Sulphate; ICH = Immunohistochemical; DAPI = 4′,6-diamidino-2-phenylindole; MHC-I = Major histocompatibility class I complex; SEM = Scanning electron microscopy; 5FU = 5-fluorouaracil; hDAM = Human adipose tissue; CLS = Capillary-like structures

INTRODUCTION

Breast cancer (BC) is globally the second most invasive cancer with a higher incidence among women[1] and the highest mortality rate in female population, making it a major socio-sanitary problem. In recent years, progresses in the diagnosis and early treatment of cancer have improved the prognosis and quality of life of many patients. However, the appearances of metastases, resistance to therapy, and/or tumor relapse remain major limitations that prevent success in treatment. Therefore, there is an increasing need to improve the understanding of the molecular mechanisms that occur in the tumorigenic process, as they may allow the development of new drugs or strategies that favor the therapeutic intervention of advanced BC.[2]

The importance of the tumor microenvironment (TME) in regulating cell behavior of BC is widely recognized.[3] Cancercells, including a small subpopulation of cancer stem cells (CSCs),which have the capacity for self-renewal, differentiation, and tumorigenicity,[4,5] co-exist with a population of stromal cells (such as fibroblasts, mesenchymal stem cells, immune cells, and vascular structures) surrounded by a matrix, forming a tumorigenic and heterogeneous niche, very favorable for tumor development. However, growing evidence suggests that noncellular components of the niche, especially the protein composition and biomechanical properties of ECM, also play a key role in the progression and metastasis of BC.[6]

The ECM in the mammary tissue constitutes a dynamic scenario of protein components, which provides mechanical support to the cells, regulates the homeostasis of the tissue, constitutes a reservoir of growth factors and cytokines, and provides tensile strength to the tissues.[7] But beyond being a simple structural and mechanical framework, it is a potent modulator of key cellular functions,[8] in addition to a physical barrier affecting therapeutic effectiveness. Although there are not evidences about the relationship between changes in ECM as cause of BC, however it has been found that they can amplify and promote growth and progression.[9] The ECM in BC tissue is deregulated and disorganized by the interactions of cancer cells and tumor stroma, establishing a "new" homeostatic balance that reflects a high interstitial pressure, mechanical stress, contractile markers and cellular rheology enhanced by a reaction similar to fibrosis called desmoplasia.[10] As such, tumor heterogeneity and the changes generated in ECM mark significant events in tumor progression, and increase cancer cells chemoresistance.[11] However, due to these complex interactions it makes it difficult to understand the roles of the ECM, which poses numerous challenges to be addressed. Recently, the best knowledge about how the components of ECM affect cancer development has led to great achievements in the recreation of biomimetic cancer microenvironments.

The great advance in biomaterials science has made it possible to imitate an *in vitro* microenvironment closer to the tumor, compared to the reductionist version of 2D cultures in monolayer.[12] The use of native ECMs as natural biomaterials and their synthetic alternatives is a very versatile tool in the investigation of signaling pathways and mechanisms underlying the wide variety of cancer cell phenotypes and functions, including drug resistance.[13] Nevertheless, despite the remarkable potential, the rational design of these biomaterials continues being very limited due to the

biochemical diversity and specificity of the BC.[14] Therefore, it is necessary to continue evolving towards an ideal model that recapitulates all the mechanistic enigmas that BC tackles.

Because of recent advances it has been possible to obtain a reconstituted *in vitro* ECM known as a decellularized ECM (dECM).[15] The dECMs not only represent a promising potential for regenerative medicine, but also these platforms have generated a substantial impact in the field of 3D biomimetic tumor-ECM models providing great advantages over the current *in vitro* models. The dECM provides a robust platform for recapitulating key tumor characteristics from improved characterization methodologies and techniques that preserve the components of ECM for more realistic modeling of cancer microenvironment.[16] The variability of sources of these dECM and their different applications such as hydrogels, bioinks hybrid scaffolds, have had great achievements in BC research and therapeutic screening.[17]

This review is focused on 2 closely related areas where the importance of ECM on BC progression may have an increasing impact on the development of new 3D *in vitro* BC tumor models. First, we highlight the importance of tumor ECM and include a specific description of how its biochemical and mechanical properties are affected in cancer development. Then, we explore the importance of biomaterials in the recreation of mimetic BC environments, with a special focus on the attractive dECMs, Including obtaining and characterization strategies. Finally, dECMs applications and clinical impact on the recent advances in BC research are discussed.

THE EXTRACELLULAR MATRIX (ECM), A PREDOMINANT ECOSYSTEM IN TUMOR PROGRESSION

The composition and dynamic organization of tumor ECM differs from normal tissue and they are determining factors in cellular and tumor behavior and development.[6] In addition, rigidity and density contribute to the transformation toward the malignant phenotype.[18] ECM in the TME also modulates intratumor signaling, providing physical and chemical signals to tumor cells, which influence cell survival, immunogenicity, angiogenesis, cell mechanics, chemoresistance, as well as in the sensitivity of cells to treatment[19] (Fig 1). In addition, the accumulative hypothesis suggests that ECM is an ambivalent non-cellular component of the stem cell (SC) niche.[6] Contact with ECM is required to preserve the capacity for self-renewal and differentiation in heterogeneous SC lineages in response to therapeutic treatments,[20] while the loss of contact with ECM would suppose an imbalance between them, losing the identity and the number of SC in different systems.[21] This alteration supports the highly chemically resistant mesenchymal phenotype and can lead to the generation of cancer stem cells (CSCs), helping to promote the invasion and metastasis of tumors to their distant sites.[22] Therefore, ECM could be an important contributor to CSC niche.

ECM. composition and contribution in BC. The mammary stromal ECM is composed of a basal membrane and interstitial matrix, formed mainly by a collection of proteoglycans, fibrous proteins, and glycoproteins, which have been investigated as key regulators in cancer progression[23] (Table 1). Collagens are the most dominant structural proteins in ECM architecture, comprising 28 different types.[24] They provide structural support to the mammary gland and affect the mechanics of the tissue, and mediate drug resistance in different tumor models.[25–27]

Fibronectin (FN) is a noncollagenous matrix glycoprotein key to cell adhesion and migration. FN regulates the epithelial-mesenchymal transition (EMT) induced by the transforming growth factor-beta (TGF-β)[28] and it is a modulator of the BC cell (BCC) invasive phenotype.[24,29,30] Laminins (LM) are the most abundant glycoproteins in the basement membrane and regulate BCC migration and metastasis[31] (Table 1). Proteoglycans (PG) confer mechanical resistance and hydration to tissues and actively participate in intercellular communication and signaling, interacting with different regulators through glycosaminoglycan (GAG) chains.[30] On the other hand, and although not less important, matrix proteins are glycoproteins that do not directly contribute to the structure of ECM but are considered powerful modulators of cell-ECM interactions. For example, the expression of tenascin C (TNC) at the invasive limit of the breast tumor predicts metastatic relapse and poor survival.[28] The overexpression of osteopontin, known as secreted phosphoprotein 1 (SSP1), promotes the development of breast tumors and favors the metastatic potential in rodent models.[32] Also, periostin (POSTN) is involved in the development and metastatic dissemination of BC and is associated with a gene expression signature, with a poor clinical outcome in anthracycline treatment.[9,33] Moreover, it affects the rigidity of ECM, a factor synergistically related to tumor progression.[34] The expression of the thrombospondin family (THBS) in BC cells themselves induces cell migration and promotes cancer invasion although THBS also display anti-angiogenic functions that leads to tumor growth inhibition in BC models.[35] Finally, Secreted Protein Acidic and Rich in Cysteine (SPARC) has a strong expression in BC

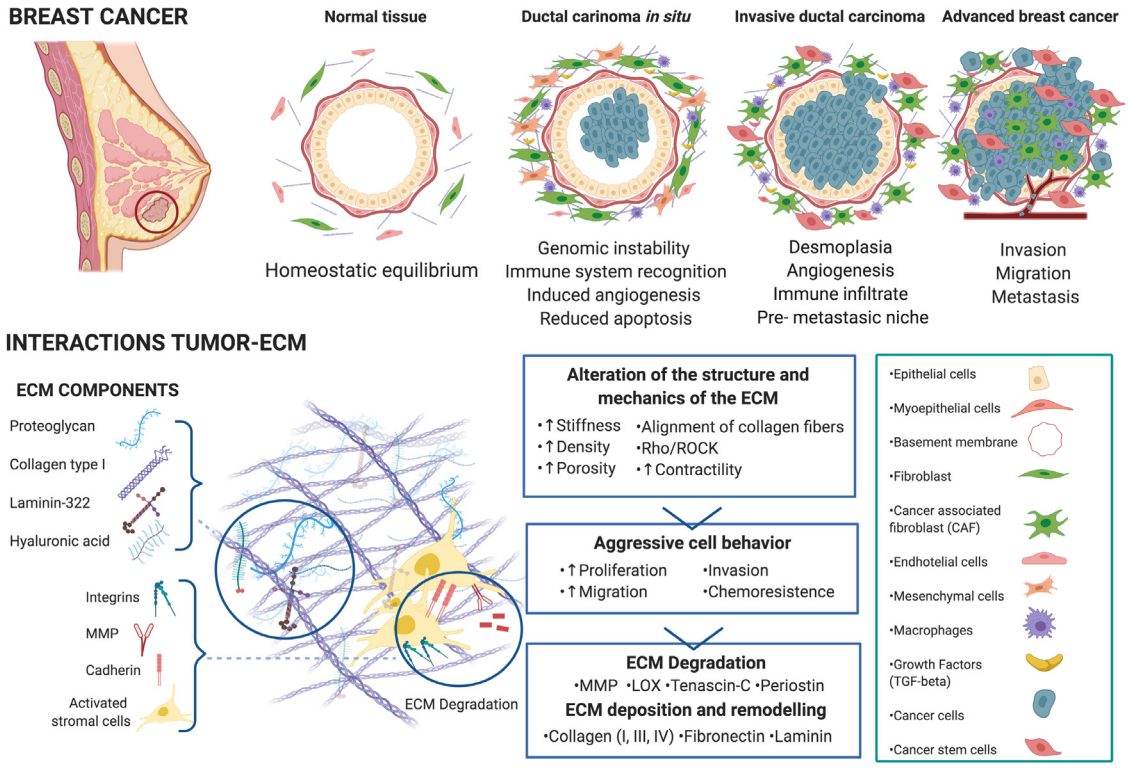

Fig 1. Schematic representation of the tumorigenic phenomena associated with each stage of the BC and the existing relationship of the main changes that occur in ECM. The deregulation of the functionality and dynamic nature of ECM, by tumor-stroma interaction, leads to the transformation of the aggressive phenotype of cancer cells and tumor-associated cells. These characteristic alterations of solid tumors are increasingly recognized as an object of study to understand the more complex mechanisms of tumor progression and the development of new therapies. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

compared to healthy breast tissue.[36,37] In short, the macromolecular composition of altered ECM has become a recognized fact in tumor progression and resistance to treatment (Table 1). These promising examples may point to major therapeutic treatments such as cancer therapies.

Moreover, BC ECM provides as a reservoir of growth factors and cytokines that are released into the TME during ECM degradation, leading multiple tumor hallmarks. For example, tumor ECM is responsible for modulating basic immune cell behaviors, including cell infiltration, differentiation, activation,and proliferation.[38] In addition, ECM drives capillary morphogenesis by release of pro- and antiangiogenic factors, which stimulate endothelial cell migration, growth,and survival.[7,18]

ECM. remodeling during tumor progression. During BC development, the ECM is deregulated and disorganized and suffers a reaction similar to fibrosis called desmoplasia.[10] This desmoplastic remodeling is characterized by an increase in the deposition and reticulation of the collagen fibers, giving rise to an increasingly rigid, dense and fibrotic ECM.[39] The excessive production of fibrillar ECM proteins has been correlated with the progression and chemoresistance of invasive cancers such as BC,[40,25–27] as it promotes proliferation,[30,41,42] cell contractility and chemotherapeutic resistance[30] (Fig 1). Collagen fibers thicken and line up perpendicularly on the invasive front of the tumor.[43] These alterations in the collagen fibers are called "tumor-associated collagen signatures" (TACS) and have been shown during breast tumor progression *in vivo.* [8] Besides, ECM alignment also affects cell morphology, where more aligned and elongated cells migrate with greater speed.[6,8,41,42] Additionally, increased rigidity in ECM can activate T cells by promoting functional integrin-mediated adhesion complex[38] and also favors the angiogenic process, improving the signaling of VEGF by encouraging the growth of endothelial cells and affecting their motion dynamics.[6,44] Moreover, fibrotic tumor ECM can promote chemoresistance. This physical barrier affects the efficacy of the drug through an intratumor blockade, because the dense and rigid tumor ECM prevents drugs

Table 1. ECM components in BC

ECM components	Function	Influence on BC	Refs
Collagens	• Provide structural support • Maintain tissue polarity	• Promote tumor progression and aggressive cell transformation • Regulate angiogenesis • Promote drug resistance	3,24,25,40,143
Fibronectin	• Mediates cell adhesion and migration	• Regulates EMT • Promotes BC invasive phenotype	24,28,29,144,145
Laminins	• Mediate cell adhesion • Maintain the polarity of the epithelium	• Regulate BCC migration, invasion, and metastasis.	
LM-111		• Induces cell-cell adhesion • Inhibits BCC spread	146
LM-332		• Enhances BCC aggressiveness • Induces BCC invasion and migration.	31
LM-511		• Mediates BCC invasion and metastasis • Enhances SCs phenotype	40,147
LM-411		• Enhances BC proliferation	148
Proteoglycans	• Regulate mechanical resistance and hydration of tissues • Participate in intercellular communication and signaling	• Regulate BC invasiveness and metastasis • Regulate tumor progression	30
HSPGs		• Enhances BC metastasis and invasive phenotype • Enhances chemoresistance	149,150
GPCs		• Regulates EMT, acting as metastasis suppressor • Enhances BC progression	151–155
GPC - 1		• Enhances BC progression	151–155
GPC-3		• Regulates EMT, acting as metastasis suppressor	33
GPC-4		• Regulates EMT by increasing the expression of metastatic BCC	154
HA		• Enhances ECM shiftiness • Enhances BC invasion and metastasis • Enhances BC progression	60,156
Versican		• Modulator of cellular behavior in BC • Induction of BCCs markers (ALDH1, CD44, integrin-β1 and Oct-1)	157,158
SLPR (Decorin, Lumican)		• Regulate BC development and angiogenesis • Metastasis suppressor	9,28,30,159
Matricellular proteins	• Modulate cell-ECM interactions and signaling pathways	• Promote BC metastasis and progression • Affect ECM rigidity	
Tenascin		• Source of premetastatic niche • Enhances BC metastasis	28,160
Osteoporin		• Promotes BC progression and metastasis	32
Periostin		• Promotes BCC metastatic dissemination • Affects ECM rigidity	9,33,34
Thrombospondine		• Promotes BC migration and metastasis • Antiangiogenic fuction	35
SPARC		• Inhibits BCC metastasis	161

from spreading from the vasculature to cancer cells.[22] In addition, it has been revealed that the accumulation of mechanical stress plays an important role in the availability of drugs, so that it can put pressure on the collapse of blood vessels, restricting the flow.

One of the contributors to ECM remodeling and therefore to the aberrant behavior of the ECM is the tumor stroma[45] (Fig 1) composed of several tumor supporting cells. Cancer-associated fibroblasts (CAFs) are the most abundant cell types and are primarily responsible for the increased deposition of ECM components and proteolytic remodeling of ECM.[46,47] CAFs mostly control the increased rigidity of the ECM and FN assembly.[8] Mesenchymal stem cells (MSCs) increase the density and alignment of fibers around them promoting more-invasive BC phenotypes.[48] Tumor-associated macrophages (TAMs) generate an immunosuppressive environment that supports the production of cytokines and proteases responsible for tumor growth, angiogenesis and metastasis.[3,49,50] Finally, endothelial cell signaling mediated by VEGF, is primarily responsible for the angiogenic process, promotes tumor formation in the early BC stage, plays a key role in vascular homeostasis and enhances the metastatic process,[51] with the consequent ECM degradation.[3,45]

Matrix metalloproteinase (MMPs) are the main zinc-dependent endopeptidases that degrade a variety of components (eg,collagen, fibronectin, laminin, tenascin) as well as cell surface growth factor receptors (eg, EGFR).[52] The combination of MMP and lysyl oxidase (LOX), increases the invasive behavior of BCCs, and initiates the reticulation of collagen, improving the rigidity of the ECM and regulating the mechanisms of ECM-cell adhesion.[8]

BIOMIMETIC STRATEGIES BASED ON ECM COMPONENTS TO RECAPITULATE THE BC MICROENVIRONMENT

In recent decades, the rational design of natural and synthetic biomaterials has enabled progress in the research about how the components of ECM contribute to the progression of BC[53](Table 2), as they better mimic the architectural complexity of the TME *in vitro.* They also provide an alternative to animal models to study the cellular behavior of cancer cells and the response to the drug, allowing the development of new diagnostic strategies.[13]

Hydrogels have become powerful tools available to mimic the key features of ECM. Although they are mechanically more unstable, they are the most widely used models by cancer researchers, as they provide a highly aqueous environment similar to that of ECM with excellent porosity and viscoelasticity properties, facilitating diffusion and drug delivery, and obtaining a more accurate recapitulation of tumor complexity and heterogeneity.[54] Additionally, the protein composition of the ECM is very relevant in the invasion of neoplastic cells.[13] Natural matrices such as collagen hydrogels facilitate the individual migration of BCCs with greater protrusion and spread than cells in reconstituted basement membrane gels (rBM or Matrigel), where cells migrate collectively without protrusion.[55] In particular, Matrigel is the most widely used matrix in BC modeling. It is composed of a set of basement membrane proteins and growth factors including TGF-β, Epidermal Growth Factor (EGF), and Platelet-derived growth factor (PDGF),[56] and rBM hydrogels allow in vitro recapitulation of tumor behavior in the in vivo cell environment.[13] In fact, these hydrogels were used in the presence of BC 3D modeling systems to improve the growth of spheroidal cultures, which resemble tumor conditions in vivo.[57] This platform can be used to study the synergy between fibroblasts and cancer cells and has also been considered a good tool for drug dissemination.[58] Interestingly, the sensitivity of MDA-MB-231BCCsembedded in Matrigel gels in response to the drug doxorubicin (DOX) was significantly higher compared to single-layer 2D cells.[59] This study demonstrated the importance of therapeutic screening in 3D in vitro models. Similar to Matrigel and Col I, the hydrogel of hyaluronic acid (HA) is an attractive biomimetic material as it is a natural GAG useful for BC tumor modeling.[60] Studies by Suo *et al.* (2019) revealed that MCF-7 BCCs grown in HA gels increased their migration and invasion capabilities with increased levels of expression of VEGF, IL-8,and FGF-β.[61] In addition, these hydrogels present a remarkable potential in drug screening assays. For example, studies by Baker *et al.* (2017) demonstrated that doxorubicin diffusion was decreased in BC cell lines T47D and MCF-7 embedded in HA hydrogels, but BCCs showed a high expression of the multidrug resistance protein (MDR1).[62]

BC ECM-based models often include a combination of multiple components, to achieve optimal conditions that more accurately reproduce the tumorigenic niche. For example, Col I and alginate hydrogels have been used to generate tumor matrices, allowing the study of the invasive behavior of BC cell lines, such as the MDA-MB-231.[63] Matrigel has also been combined with alginate to control its stiffness.[54]Cavo *et al.*(2018) established a formula (i.e. 50% Alginate, 50% Matrigel) that resembled BCCs phenotype and invasion capability observed *"in vivo."*[64] Moreover, Stowers *et al.* (2017) developed a hydrogel platform consisting of Matrigel/alginate containing light-sensitive liposomes

Table 2. Natural and synthetic biomaterials

Biomaterial	Advantages	Disadvantages	BC related research	Refs
Natural Biomaterials				
Matrigel	• Rapid encapsulation • Biologically active • Efficiency in acinar spheroid formation • Controls the behavior and morphology of cancer cells	• Batch-to-batch variability • Difficulty of setting parameters independently • Limited cross-linking to biocompatible catalysts • Uncontrolled degradation • Poorly defined	• Matrigel increased the invasive phenotype of BCCs in co-culture with hMSC cells and stromal fibroblasts • MDA-MB-231 treated with DOX were more sensitive at Matrigel	131 162 163 67 60
Col I	• Fibrillary system • Low cost • Processing easy • Bioactive binding points • Migration, invasion and modeling of BC in metastasis	• Low elastic module • Reduced density • Temperature and pH-sensitive • Fiber alignment • Lack acinar structures	• MDA-MB-231showed stiffness-dependent apoptosis and migration in collagen matrices • Docetaxel increased the invasion potential in MDA-MB-231 and HCC1806 embedded in collagen gels	164 11 165 13 60
Hyaluronicacid(HA)	• Covalently reticulated • Spontaneous crossing • Moldable mechanism • Fast diffusion • Bunch formation • Promotes tumorigenic signaling by CD44	• High molecular weight • High viscosity • Affects cell signaling and tumorigenesis	• MDA-MB-231 showed greater invasiveness towards EGF. Invasion decreased in the presence of cetuximab • MCF-7 and T47D showed increased resistance to DOX through MDR1 flow	166 167 57 62 54 60 168
Hybridbiomaterials				
Matrigel/ Alginate	• High reticulation • Modulation of mechanical properties	• Modifications can affect cell signaling • Changes the density of the cell adhesion ligand	• High rigidity induces malignant phenotype of normal breast epithelium • MDA-MB-231 were more resistant to DOX in the rigid hydrogel	66 169 67
GelMA	• Stable hydrogel • Pore formation control • Better reticulation • Low cost • Low cytotoxicity • Rich in adhesion ligands	• Dependent on reticulation agents • Susceptible to degradation • *In vivo* biocompatibility	• Higher proliferation rate at 10% of GelMa hydrogels • MDA-MB-231 and MDA-MB-468 showed more resistance to PTX by reducing the response to taxanes	70 58 170 54
Synthetic Biomaterials				
PEG	• Reproduction • Modified surface • Self-assembly of cellular acini • Biocompatible • Large-scale use • Direct cross-linking with adhesive and degradable ligands • Easy encapsulation and functionalization	• Loss of bioactive sites • Bioinert • Low interaction with cells • Exempt from protein absorption • Nonbiodegradable • Without acinar structures	• Favor the growth and tumorigenic signaling of the mammary epithelial cells • PEG system combined with phosphorylcholine (PEG-PC) showed resistance to sorafenib	171 172 173 67 60
PLGA	• High porosity • Biodegradables • High mechanical resistance • *In vitro* biocompatible	• *In vivo* biocompatibility • Low cell affinity • Poor cellular response • Hydrophobic	• MCF-7 grown on PLGA microspheres were more resistant to doxorubicin after cryopreservation	73 167 174

PTX paclitaxel; DOX doxorubicin.

enclosed in Ca^{2+}, which were activated to release the Ca^{2+} and to reticulate the gel in a controlled way. They found that nonmalignant MCF-10Amammary epithelial cells developed a more invasive phenotype when grown in rigid hydrogels, and the hydrogel stiffness was increased by inducing the invasiveness of the cells.[65] A similar model was used to examine the rigid properties of normal and tumor breast tissue, and it was found that the rigid environment of ECM can modulate drug resistance. MDA-MB-231 cells grown in rigid hydrogels were shown to be three times more chemically resistant than in soft hydrogels.[66] All these studies strongly support the use of ECM-derived models in BC research, as they provide a good physiological basis and facilitate a better understanding of the behavior of cancer cells. However, the ability of each of these materials to control the biomechanical properties of the scaffold is poor, due to uncontrolled degradation, batch-to-batch variability, virtually low viscoelasticity (specifically of collagen and Matrigel),and lack of bioactive adhesion points.[67] Therefore, this fact leads to confusion about the real effect of ECM on cell migration and propagation.[68]

Another class of biomaterials that are increasingly being used as 3D matrices for BC, due to their ideal biochemical properties and biocompatibility, are biomaterials derived from natural polymers such as chitosan, silk fibroin, and agarose. Nonetheless, they require chemical functionalization because they lack points of cellular adhesion and, therefore, also prove to be limiting when studying cellular mechanisms.[69] Furthermore, the functionalization of 3D scaffolds such as methacrylate gelatin (GelMA) is increasingly significant since it combines the hydration and bioactivity properties of gelatin with the mechanical characteristics provided by the methacrylate component. GelMA hydrogels have become an object of attention for modeling the key characteristics related to BC tumor progression, by showing increased invasive and tumorigenic capacity *in vitro*.[70]Moreover, hydrogels can be made of synthetic materials. They are mechanically more resistant and offer the possibility of a more accurate assessment of biochemical characteristics in tumor ECM models.[69] For example, cells encapsulated in polyethylene glycol (PEG) hydrogels preserve viability and deposit their own ECM (GAG and collagen).[71] Additionally, a combination of different hydrogel platforms (PEG-maleimide [PEG-MAL], PEG-phosphocholine [PEG-PC], polyacrylamide [PAM]) was used to compare the resistance of the BC cell lines MCF-7 and SkBr3 to drug inhibitors and cytotoxic agents.[72] The results indicated that the response to the drug varied according to biomaterial, stiffness, dimensionality and, point of adhesion to the cell.[58] The combination of poly-lactic acid (PLA) and poly-glycolic acid (PGA), to form poly lactic-co-glycolic acid was also used as a platform for BCC culture. Interestingly, poly lactic-co-glycolic acid was used in the culture of MCF-7 cells for the cryopreservation of3D tumor models using a water/oil emulsion.[73]

However, the lack of endogenous factors and the difficulty of remodeling by the cells, make these biomaterials not enough suitable to reconstitute the native scenario where the cells migrate through the matrix.[74] Despite the remarkable potential, biomimetic biomaterials of ECM, as well as synthetic alternatives used so far cannot fully reproduce the biomechanical and biochemical complexity of tumor ECM. Therefore, it is difficult to mimic a global interpretation of how ECM drives the biological functioning of cells and exploits this capacity for various therapeutic applications. This is why the field of biomaterials must continue to evolve and incorporate new options that allow such complexity to be imitated with greater precision.

DECELLULARIZED EXTRACELLULAR MATRIX (DECM) AS A NEW APPROACH TO CLARIFY THE ROLE OF THE BC ECM MICROENVIRONMENT

Given the need to improve the understanding of how ECM regulates the behavior of cancer cells, reconstituted *in vitro* ECM known as dECM has acquired greater relevance as novel platforms. In fact, several proteomic studies have been conducted using these dECMs, which have profoundly clarified the composition and pathological alterations of the ECM in tumor and metastatic niches (Table 3). These investigations on healthy tissue and BC tumor tissue ECMs have been used to identify the role in tumor ECM generation by stromal and/or tumor cells,[75–78] describe the differences between different healthy and tumor ECMs,[75,78–80] to elucidate new functions of tumor ECM,[77,81] to find protein-level differences between low and highly metastatic carcinomas,[75] and to identify new potential prognostic and diagnostic biomarkers.[75,79,81,82] The diversity and heterogeneity found in the composition of healthy, tumor, or premetastatic ECM emphasize the importance of recapitulating the whole set of BC ECM components to study tumor behavior, rather than individual ECM proteins.

In this context, the isolation of dECMs has allowed the generation of tumor models that facilitate the recapitulation of key ECM characteristics and interactions between ECM, cancer cells and stromal populations associated with TME, providing significant advantages in the area of 3D *in vitro* tumor modeling.[14] Furthermore, the dECM, as a biomaterial, represents a more

Table 3. dECMs platforms to study BC ECM nature

ECM source	Contribution	Refs
Breast carcinoma xenografts	• Description of the role of tumor and stromal cells in ECM regeneration • Description of the differences in the ECM composition of tumors with different metastatic capacity • Identification of the causal role in metastasis of distinct proteins • Identification of potential prognostic and diagnostic biomarkers	75
ECM of brain, lung, liver, and bone marrow metastases of triple-negative BC	• Description of the role of tumor and stromal cells in ECM regeneration and metastatic niche. • Identification of specific proteins (SERPINIB1) implicated in metastatic tropism	76
ECM of tumor mammary glands	• Contribution of the ECM in metastasis. • Identification of specific proteins (ColVI) that promote BCC invasiveness	81
ECM of mammary gland and liver of rodent models	• Description of the differences between ECMs of different origin. • Study of the alteration of the ECM mammary gland throughout the reproductive cycle.	79
ECM of healthy tissue, primary tumor, and metastases in lymph nodes and lungs of BCCs	• Studies on the compositional change of tumor ECM and healthy tissue • Description of the differences in protein composition and abundance between metastatic sites	80
ECM from co-culture of BCCs, adipose derived stem cells, and fibroblasts	• Describe the role of the cytokine CLL5 in BCC invasion through the generation of ColIV in the tumor stoma.	77
ECM from lungs and livers containing metastatic tumors	• Identify novel ECM proteins associated with colonization	78
ECM from primary breast cancer tumors	• Analyze protein expression patterns among BC patients.	82

reliable and favorable model with respect to those previously discussed, since it possesses better biocompatible characteristics and provides the architecture and mechanical integrity of the native ECM while avoiding the unfavorable biological and immunological responses that can be caused by cellular and nuclear biomaterials.[83] Recent advances in decellularization methods have become a key focus for preparing more suitable cell-derived and tissue-derived dECM models.[15] In recent decades, dECM models have been used for the development of hydrogels, hybrid platforms or bioinks for 3D bioprinting,[84] with considerable success in BC research and the pharmaceutical industry [15](Fig 2). In the following sections we highlight the most widely used methodologies for dECM obtaining, characterization, solubilization and gelification.

Tissues/organs-derived ECM and cultured cells-derived ECM. BC dECMs can be obtained from 2 different types of sources, directly from cells grown *in vitro* (cultured cells),[15] or through tissues and organs, commonly adipose tissue, as cellular responses are enhanced when used for 3D matrix design[85] (Fig 2). dECMs derived from cells such as MSCs, fibroblasts or embryonic stem cells (ESCs), are a promising strategy, although it needs more intensive optimization, due to the low yield they show compared to tissue decellularization.[14,86,87] Cell-derived dECMscan be obtained directly through *in vitro* cell culture and expanded on a larger scale.[86] However, it has limitations in accurately mimicking the composition and the architectural and biomechanical properties of the native ECM, because it can be altered by several factors associated with culture conditions (eg,culture medium, cell type, initial substrates, turns number,and culture periods).[15] Therefore, cell-derived Decm turns out to be slightly different from native ECMs. Furthermore, it can reintegrate native ECM as a SC niche, as dECMs can induce specific SC functions and preserve them in their undifferentiated state *in vitro*.[16] For example, cell-derived Decm was shown to be compatible with growing and differentiating MSCs by preserving the same proliferative capacity after multiple passages, which is of relevant biological importance for mimicking the tumor dynamics of ECM.[88]

Alternatively, tissue/organ derived dECMs are interesting to better recapitulate native ECM because the structural and mechanical characteristics are expected to be more similar to those of the native tissue ensuring homeostasis and regeneration. The disadvantage of tissue/organ Decm-based platforms is the limited material supply, which is insufficient after decellularization, for

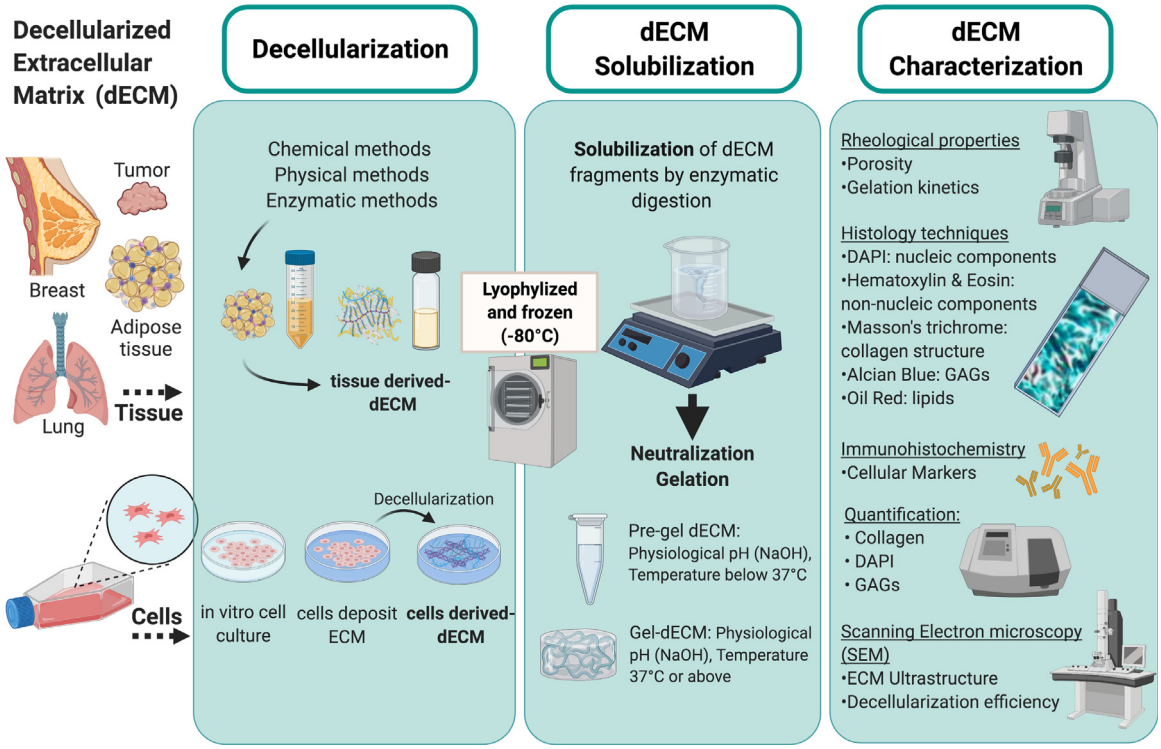

Fig 2. Schematic representation of the main steps followed to obtain dECM and its applicability *in vitro* and *in vivo*. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

clinically relevant applications. The obtainment from tissue/organ Decm in limited regions, as bone marrow or SC niches, can be complicated, as these regions are difficult to isolate.[16] Tissue-derived Decm presents problems when analyzing the intracellular signaling using large-scale cells and methods that reduce the available sample, which does not occur in cell-derived dECMs. The heterogeneous nature of the ECM and tissue specificity also play a role in the development of Decm platforms, as the batch-to-batch variability of tissue/organ-derived Decm is very wide, even among patients with the same cancer.[16] So, working with this type of matrix can be a complete challenge. Therefore, cancer research requires careful consideration in the correct selection of dECM sources.

Decellularization: a key process for preserving the components of ECM. The initial step to obtaining a reconstituted *in vitro* ECM is the process of decellularization, whose objective is to efficiently eliminate all cellular and antigenic material, conserving the essence of the ECM, that is, preserving the composition and microstructure of the matrix complex and reducing the immunogenicity of the biological scaffolds.[84,89] The decellularization process is commonly initiated by cell membrane lysis, followed by separation of the ECM cellular components. Researchers have already designed numerous protocols to eliminate these cellular components,[90] and generally, combinations of different methods are used, which have been classified into 3 types: physical, chemical, and enzymatic (Fig 2). Physical methods disrupt the membrane and release the cell content through a series of treatments such as agitation, sonication, pressure, or freeze-thaw cycles.[17] For chemical decellularization, detergents are the most commonly used chemicals, such as sodium deoxysulfate , Triton X-100,or Sodium lauryl Ether Sulphate .[91] These detergents lyse the lipid membranes but can modify the ECM microstructure by reducing the mechanical stability and the loss of ECM components such as GAGs.[89] Alkaline and acidic solutions are also used in chemical treatments, being efficient in removing cytoplasmic material and degradation of nucleic acids, and can preserve the structure and function of growth factors such as TGF-β.[92] However, they can have adverse effects on GAGs.[16] Polar extraction solvents such as isopropanol, or ethanol have also been frequently used to remove lipids from fatty tissue,[93] a source for dECM that has received much attention because of its abundance of ECM components, such as elastin, GAGs, laminin, cytokines and factors that regulate many cellular processes, but has a high lipid content that can hinder the process of decellularization.[94]

Proteolytic enzymes such as trypsin, along with nucleases and chelating agents are widely used in enzymatic methods, which act by hydrolyzing protein peptide bonds, and removing specific dECM molecules. However, prolonged use of these enzymes can cause damage to the components of the ECM, as well as alterations in the content of collagen, elastins, or GAGs.[95] Lipases and colipases have also been frequently used to catalyze the hydrolysis of lipids. Nucleases such as DNAse and RNAse efficiently remove nucleic acids, leaving behind cytosolic components.[16] On the other hand, the methods of sterilization and lyophilization, used after the process of decellularization for later use, should be standardized, since the matrix samples during conservation and storage can be affected in terms of ultrastructure, biodegradation, and remodeling of ECM.[96]

Solubilization and gelification of the dECM. An important aspect after decellularization is the solubilization and gelification of dECM at optimal temperature and pH ranges that ensure the formation of dECM hydrogel. This collagen-based self-assembly process has also been shown to depend on the presence of GAGs, proteoglycans, and ECM proteins. So, the biochemical composition of the native source and the proportion of proteins remaining after decellularization may be factors affecting polymerization kinetics.[97] Also, the selection of the solubilization protocol is fundamental in order not to affect the formation of the dECM hydrogel.[98]

Solubilization of dECM is performed by enzymatic digestion usually with acid pepsin, an enzyme derived from porcine gastric juices that are responsible for breaking down the collagen fibrils without affecting the ultrastructure of the collagen and solubilizing most of the acid-insoluble collagen. However, treatment with pepsin can damage the biochemical composition of the ECM, inducing biodegradation and, therefore, the loss of molecular components, an unfavorable option since the assembly of collagen fibers are affected.[98] Because of that, new strategies have been proved, as α-amylase or urea solutions,[99–102] less aggressive than pepsin digestion. The digestion period must also be adapted for each application,[103] as well as some parameters, such as temperature, pH or concentration, for improved control of ECM hydrogel mechanics.[104]In contrast, self-gelling hydrogels (gels controlled by their properties), although it can mimic the composition and architecture of the native ECM of rat and human breast adipose tissue, these soft platforms are more susceptible to degradation than their native ECM counterparts.[105]

Neutralization at physiological pH and *in vitro* temperature is a process called collagen kinetics, and allows the spontaneous reform of the intramolecular bonds of the monomeric components into a homogeneous gel.[106] The solubilized dECM is neutralized at a physiological pH with NaOH under cold conditions to prevent instant gelation, as an acid or alkaline pH can have an unfavorable effect on the encapsulating cells.[98] This pH-adjusted, heat-sensitive dECM solution is called a dECM pre-gel and is transformed into a gel-dECM when the temperature rises to 37°C. After gelling is complete, the gel structures are stable, after the complete polymerization. The use of dECM hydrogels has proven to be successful in a variety of clinical applications resulting in major advances in 3D bioprinting technology. However, this digestion process is not strictly necessary. After decellularization, cells can be seeded directly onto dECM scaffolds without the requirement of solubilizing and gelling agents. This has been observed by Wishart *et al.* (2020) used dECM scaffold as a model for cell migration studies,[81] and Liu *et al.* (2018), where dECM scaffolds were seeded with MCF-7 cells to demonstrate the process of cell recellularization *in vitro*.[107]

Characterization of dECM. The efficiency of decellularization can be checked by a set of characterization tests, such as histological, immunohistochemical (ICH), and other less specific techniques such as colorimetry, solubility, and absorbance methods (Fig 2). The presence of nucleic components can be observed using cell nuclei stains, such as DAPI (4′,6-diamidino-2-phenylindole) or Hoechst.[17] DNA quantification is another important parameter to measure using spectrophotometry (DNA absorbance of approximately 260 nm), which estimates dry weight loss during enzymatic treatment.[108] In addition, histological staining is the fastest the most commonly used method to evaluate the efficiency of cell material removal,[109–111] and allows direct testing of non-nucleic components. Other alternative stains include Masson's Trichome stain and Picrosirius red stain,[112] to characterize the collagen structure that gives resistance to ECM. Alcian blue staining detects GAG and Oil Red identifies the lipid content of dECM.[111] In other studies, the fuchsine method has been used to dye the elastic fibers of the ECM.[113] Another technique for detecting specific cellular phenotypes is the ICH that allows the identification of cellular markers such as CD31, which is an endothelial cell marker, VEGF or specific ECM protein's such as Col IV, vitronectin,or laminin.[111] In the same context, ICH is increasingly being used to quantify collagen and to detect the presence/absence of the major histocompatibility class I complex (MHC-I) whose absence is an indicative result of elimination of alloantigenicity.[111] On the other hand, other less commonly used techniques include the quantification of soluble elastin, using a Fastin test kit,[114] the

quantification of acid/pepsin soluble collagen employing a test kit (Bircol) and absorbance,[113] which may be of interest for measuring the effectiveness of decellularization methods. The evaluation of ECM ultrastructure is done using scanning electron microscopy (SEM) to confirm that the ECM has been correctly decellularized.[115] The studies of the mechanical properties include rheological analysis. For example, porosity is studied by liquid displacement using ethanol as it easily penetrates the pores without causing contraction.[109] Freeze kinetics, tensile tests, and viscoelastic properties are useful for more advanced characterization.[108] Finally, advances in novel mass spectrometry technology (eg, MALDI-TOF)[14] represent a powerful surface-sensitive analytical method for the characterization of macromolecular components of ECM[16] without requiring specific markers or the addition of an analytical matrix.[116]

APPLICATION OF DECM IN THE GENERATION OF *IN VITRO* BIOMIMETIC BC MODELS

The development of dECM from tissue biopsies has achieved a lot of important knowledge in regenerative medicine.[14] However, cancer is a heterogeneous disease and the composition of the ECM derived from cancerous tissue may differ from the normal tissue source and, therefore, cancer tissue dECM may be more appropriate for studies ranging from cancer biology to therapeutic strategies. Only recently the production of these dECM-based biomaterials has been translated for modeling *in vitro* tumor, enabling major advances in the understanding of cancer biology.

In the research developed by Liu *et al*.(2019), dECM obtained from human BC tissue promoted the EMT of MCF-7 cells, while reducing the expression of epithelial marker CDH1 (E-cadherin) and increasing the levels of EMT-related genes, VIM, ZEB1(tumorigenic promoter) and SNAIL.[107] In addition, MCF-7 cells cultured in dECM scaffolds displayed enhanced resistance to 5-fluorouracil (5-FU) treatment, and higher expression of SC markers (Oct4, Sox2, and CD49f) compared to monolayer cells. Consistently with these studies, Dunne *et al*. (2014) designed a 3D model of dECM derived from healthy human adipose tissue (hDAM) to investigate cellular responses to BC. This model showed a cell proliferation profile similar to xenograft model, when BCCs were cultured on hDAM scaffolds. Interestingly, compared to 2D and Matrigel culture, the levels of the epithelial marker CDH1 decreased, while the expression of mesenchymal cell markers (N-cadherin and vimentin) significantly increased in the dECM scaffolds, suggesting that dECM favors the EMT process. This work also analyzed the response to pharmacological treatments such as doxorubicin and lapatinib, which showed high resistance in dECM through the activation of EGFR and Akt.[115] Similarly, the results of Leiva *et al.* (2020) also indicate the influence of dECM platforms on therapeutic response. MCF-7 cells cultured in human BC tissue-derived dECM adapted to the environment and increased their resistance to 5-fluorouracil, doxorubicin and paclitaxel compared to 2D cell cultures, suggesting a diagnostic tool in treatment prediction.[117]

The comparison between normal and pathogenic tissue matrix is a strong point that is being recently studied. In this line, an experimental assay has been done to compare the cell behavior of normal dECM and dECM derived from cancerous tissue biopsies. Jin *et al.* (2019) analyzed the cellular behavior of the MCF-7 BCC and revealed that in the scaffold of cancerous dECM, MCF-7 cells showed signs of cell proliferation unlike normal dECM. Also, results showed that BC dECM exhibited a high expression of MMP-9 and promoted EMT response and tumor angiogenesis.[91] In concordance with this result, another study focused in a tumor-on-chip development that replicated BCs in vitro tumor stroma activation during tumor epithelial invasion. They reported an ECM remodeling that affects to hyaluronic acid, fibronectin and collagens, and showed the role of MMPs in this matrix remodeling, demonstrating an overexpression of MMP-9 and MMP-2.[118]

Additionally, cultured cells-derived dECMs have been recently used for the *in vitro* BC model development. Nayak *et al.* (2019) engineered a PCL scaffold functionalized by CAFs culture-derived dECM that enhanced BCC attachment and viability. Furthermore, they tested current antitumor drugs in different patient samples, showing different responses to the same drug, which reinforce the use of autologous 3D models for personalized treatments.[119] Similarly, Serebriiskii *et al.* (2008) used fibroblast dECM to obtain 3D tumor models to study the chemoprotective effect of tumor ECM.[120] Brett *et al.* (2020) developed a model from BCCs, adipose derived stem cells and dermal fibroblasts co-culture dECM in order to analyze ECM structure and functions. Importantly, they found a key role for chemokine CCL5 in the generation and organization of striated tumor collagen and, thus, in the BCCstumorigenicity.[121] Curiously, Hoshiba *et al* (2013) reported that BC lines displayed different behavior in dECM derived from BCCs in different malignancy stages, which suggests that staged tumorigenesis-mimicking dECMs are suitable for the study of ECM roles in tumor progression. In addition, dECM *in vitro* models begin to cover different BC aspects and

processes.[122]Aguado *et al.* (2016) engineered PCL scaffolds functionalized with lung and liver dECM from healthy and tumorigenic mice in order to study tumor cell colonization *in vivo*. They found that lung and liver decellularized matrix from tumor tissue coatings enhance tumor cell adhesion compared to healthy ones, and when the functionalized scaffolds were implanted in mouse models of BC, diseased dECM also enhanced tumor cell colonization.[123] Alike, Xiong *et al.* (2018) developed a model based on dECM derived from native lung tissue to mimic the metastatic colonization of BC in the lungs, one of the most frequent sites of colonization. The results showed that BC metastatic cell lines, MDA-MB-231 and 4T1, invaded and colonized pulmonary dECM.[124] In fact, lung dECMs has been widely used as *in vitro* platforms for BC research, especially for antitumor drug screening.[125,126] Interestingly, Li *et al.* (2019) performed an assay comparing common BC bioengineered scaffolds including decellularized lung, chitosan/gelatin and PLA scaffolds. They found that tumor development *in vitro* and *in vivo* was not positively proportional in PLA and chitosan/gelatin scaffolds, whereas natural-derived materials showed better biocompatibility, oncogenicity and angiogenesis.[127]

The incorporation of vascular structures into *in vitro* dECM platforms may be a crucial factor in the functional investigation of most anticancer therapies, as well as an alternative means of targeting tumor vasculature.[128] BCC cell-derived dECMs co-cultured with fibroblasts are showing potential promising results in the study of capillary morphogenesis. In this aspect, the results of Hielscher et al (2012) indicated that fibroblast-derived dECM and BCC promoted the rapid formation of capillary-like structures (CLS), in addition to elevated expression of WF, a glycoprotein involved in vascular homeostasis, and differences in the TNF-α.[129] Because of the profound role of hypoxia in tumor progression, recently it has been demonstrated that hypoxia directed morphological changes in the organization of BC dECM fibers, suggesting that hypoxic ECM altered EC responses by up-regulating the expression of pro-angiogenic factors and MMPs.[130]

Moving towards a different approach, BCCs and hydrogel bioprinting techniques can improve 3D culture systems by mimicking the structure of ductal branches. Self-gelling dECM hydrogels derived from rat and human breast adipose tissue were used as bioinks, allowing the growth of tumor organoids by culturing different BC cell lines and providing an appropriate 3D model for the study of ECM.[105]

The above-described studies show that dECM and cancer are closely related. Cancer tissue dECM is an attractive model that could provide new insights into the roles of ECM in the oncogenic process. Mimicking the spatial organization of the native ECM by assembling the ECM molecules allows a more thorough examination of the behavior of the cancer cells. dECM appears to promote angiogenesis, BC cell migration and exhibits a high expression of EMT markers. Besides, dECM represents a culture substrate for the development of anti-cancer drug platforms. In general, the dECM could be an ideal *in vitro* 3D culture model for future BC research and to establish a personalized medicine from patients' biopsies.[16]

DECELLULARIZED ECM, A PROMISING BC BIOMIMETIC MODEL COMPARED TO CONVENTIONAL SUPPORTS

Faced with the need to improve the reductionist vision of monolayer cultures, the scientific community has led the use of natural ECM materials and synthetic alternatives as 3D tumor models to better recapitulate several aspects in the structural, mechanical and biochemical context of native tissues. However, natural biomaterials have been studied under inherent limitations that make them very challenging when studying the behaviors induced by these systems.[67] For example, the batch-to-batch variability of Matrigel is difficult to quantify because the proportions of natural biomaterials it comprises are often poorly defined, impacting the cellular response that occurs in the material system.[131] On the other hand, the mechanisms of reticulation are limited to biocompatible ligands, since these gels have a narrow mechanical range.[131] Adjusting this parameter has the effect of altering the concentration of the proteins in the system. In addition, ligand density is closely associated with stiffness, depending on the amount of protein and degradable binding sites.[132] The application of these materials is easy to use thanks to their biocompatible and biofunctional nature, but so far they are still limited under experimental use, due to the lack of control in the laboratory environment.[131]

Looking to overcome the limitations of conventional strategies, dECMs provide an attractive platform to study the key communications between cancer cells and stromal populations associated with ECM. The combination of dECM and cancer cell scaffolds establishes a fundamental guide to improve the understanding of cell-mediated interactions and the design of more sophisticated *in vitro* human tumor models.[14] Ferreira *et al.* (2021) recapitulated the invasive profile of metastatic BCCs from the development of heterotypic BC spheroids and CAFs *in vitro* enriched with dECM microfibrillar fragments. In particular, these models exhibited higher expression of key biomarkers

and metabolomics, further physio mimetic potential and higher specific resistance to different chemotherapies.[133] As we have shown, recent dECM-based models already highlight their potential as valuable platforms for therapeutic screening, and their ability to reproduce all stages of the tumor.[14] dECM hydrogels can support cell behavior, providing even better properties than other substrates such as Matrigel or individual components, such as collagen.[100,134,135] For example, it has been shown that the existence of a rigid TME can affect the sensitivity of cancer cells to chemotherapeutic treatment.[66] High-stiffness dECM hydrogels exhibited higher resistance to doxorubicin compared to low-stiffness dECM and provided a cytocompatible 3D environment for studying cell viability.[136] The specific tissue composition allows the cells to maintain the phenotype and their native functions when cultured in dECM, responding in a specific way according to the source tissue.[98] Pati *et al.*(2015) showed that after decellularization, dECM scaffolds retained growth factors related to the native tissue niche and thus allowed the cells to carry out their functions.[85] Therefore, using tissue from the mammary gland as a source of dECM would allow the design of a 3D model very similar to the TME of the breast.[54] Also, dECMs promote cell encapsulation and can be remodeled by the cells.[98] The cells encapsulated in dECM show affinity to their matrix and can specifically bind, which promotes cell organization and tissue maturation.[137] Cell-derived dECMs allow the influence of ECM composition and mechanical properties on tumor progression from highly customizable platforms.[14]

The controllable nature of these platforms can be exploited for maximum efficiency in a wide variety of applications (Fig 3). Genetic engineering technology can be used to modify cell-derived ECM and to analyze the influence of a single component on cancer-cell ECM interactions.[14] Tumor models based on the recellularization from tissue-derived dECMs have been useful for the study of the adjacent microenvironment of cancer cells, where it has been shown how the dECM scaffold showed a very fibrous and fibrillary preserved microstructure.[138] This has led to an attempt to correlate ECM and disposition with cancer cell invasion and time. Similarly, the combination of dECM-based models with recent tumor-on-a-chip technologies provides

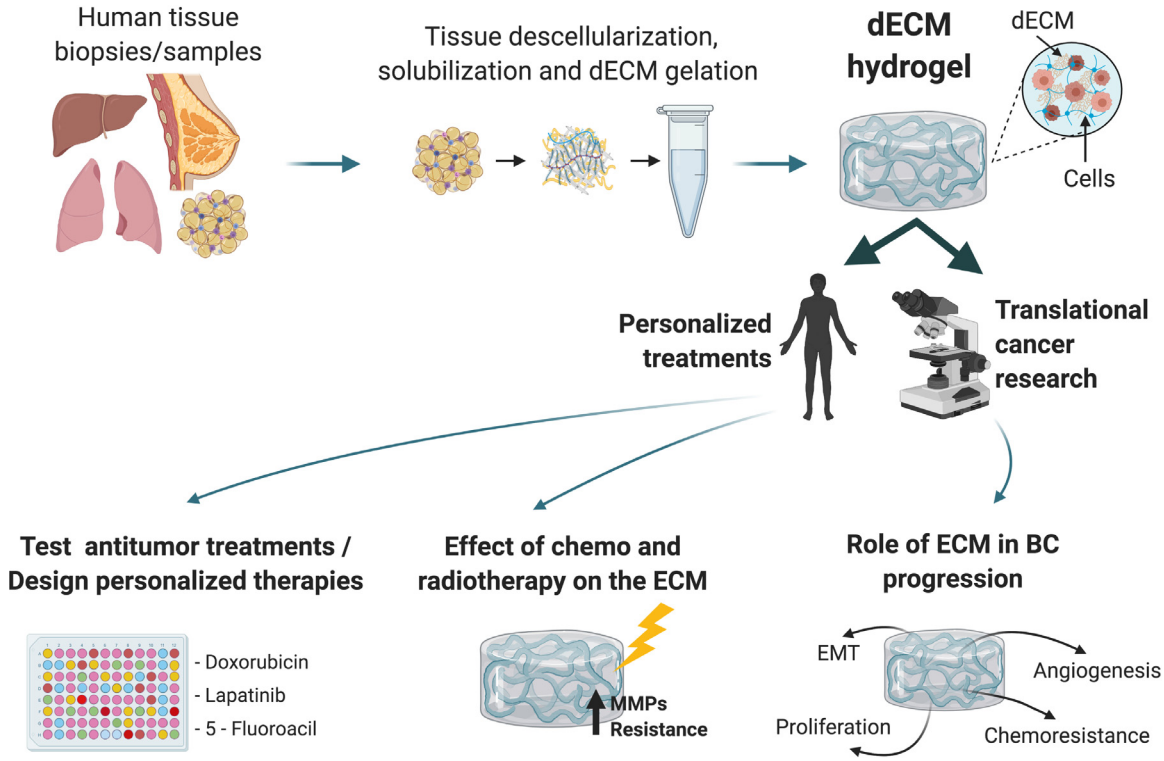

Fig 3. Schematic representation of decellularized ECM applications *in vitro* and *in vivo*. The versatility of decellularized ECMs allows them to be used in different applications from *in vitro* research of the role of ECM in BC progression, to the development of organoid models to establish a personalized therapy or study the chemo and radio therapy effect on the ECM, in order to facilitate the diagnosis and treatment of each patient. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

an attractive, higher performance platform for mimicking TME in which a combination of therapeutic strategies and a diversity of biophysical and biochemical model parameters can be achieved.[14] Consequently, dECM models may be a promising tool to evolve in the area of *in vitro* tumor modeling towards more personalized medicine for the BC patient (Fig 3). In addition, these platforms show great potential to study the effects of therapeutics in the tumor ECM. As chemo and radiotherapy are demonstrated to modify the ECM, they can also alter treatmentresponses.[19,139,140] For example, recently it have been demonstrated that radiotherapy can enhance MMPs expression, leading to BC progression.[141] Therefore, dECM models have been used to study treatment responses, considering the ECM remodeling induced by the treatment[142]and so to test new drugs targeting ECM.

CONCLUSIONS

A deeper knowledge of the functional versatility and dynamic nature of the ECM has improved the understanding of cancer biology, and it is increasingly considered as a promising therapeutic target against BC. In that context, tumor models based on dECM have become an idyllic platform to reproduce the architectural complexity of native ECM and provide a vital path to a better understanding of cell-ECM interactions. Overcoming the limitations of conventional natural biomaterials, the application of dECM in cancer research has improved our ability to recognize tumor mechanisms and evaluate specific behaviors of cancer cells, as well as the identification of new anti-cancer drugs, achieving great success in the pharmaceutical sector. Furthermore, the new approaches combined with the development of new strategies, as proteomics technologies, 3D bioprinting technology, and/or organ and tumor-on-a-chip open us the possibility of generating more sophisticated models adapted to the needs of each patient, occupying a place in BC precision medicine.

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