Review

Decellularization of tumours: A new frontier in tissue engineering

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Abstract

Cancer is one of the leading causes of death worldwide. The tumour extracellular matrix (ECM) has unique features in terms of composition and mechanical properties, resulting in a structurally and chemically different ECM to that of native, healthy tissues. This paper reviews to date the efforts into decellularization of tumours, which in the authors' view represents a new frontier in the ever evolving field of tumour tissue engineering. An overview of the ECM and its importance in cancer is given, ending with examples of research using decellularized tumours, which has already indicated potential therapeutic targets, unravelled malignancy mechanisms or response to chemotherapy agents. The review highlights that more research is needed in this area, which can answer important questions related to tumour formation and progression to ultimately identify new and effective therapeutic targets. Within the near-future of personalized medicine, this research can create patient-specific tumour models and therapeutic regimes.

Keywords

Cancer, tumour, extracellular matrix, decellularization, decellularized tumour

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Introduction

Cancer is one of the leading causes of death worldwide. In 2020, there were 19.3 million new cases and 9.96 million cancer-related deaths reported globally.[1] Because of the ageing population, it is estimated that by 2040 the number of new cancer cases per year will rise to 30.2 million and the number of cancer-related deaths to 16.3 million.[1] Therefore, great efforts are being made to overcome this deadly disease, which has mainly centred on the individual cancer cells, focusing on the genetic mutations and intracellular signalling leading to the altered cellular behaviour observed in cancer. Some of these altered cell behavioural features include loss of anchorage dependency, limitless proliferation, stimulation of migration for metastasis and chemoresistance.[2] In recent years, a massive shift occurred where scientists are regarding cancer as distinct tissues or organ-like structures, to better understand the mechanisms of cancer cells behaviour.

The various cancer tissues have a tumour microenvironment (TME) comprised of a heterogeneous mixture of different cell populations including cancerous and non-cancerous cells, growth factors and extracellular matrix (ECM), which plays a key role in regulating cell behaviour.[3–5] More in detail, the TME comprises tumour cells, tumour stromal cells like cancer-associated fibroblasts (CAFs), endothelial cells, pericytes and immune cells, for example, microglia, macrophages, lymphocytes and platelets.[6] The non-cellular component of the TME is the ECM which is composed of collagen, fibronectin, hyaluronan or laminin among other

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components. The ECM of tumours is stiffer than that of healthy tissues and this stiffness increases cancer cells proliferation and survival, and induces immunosuppression. The ECM is continuously remodelled, not only in tumours but also in healthy tissues.[5] ECM remodelling is crucial for understanding tumour progression.[5] The function of cellular and non-cellular TME components is controlled by tumour cells via complex signalling networks, resulting in tumour formation and growth, deficient response to therapy and chemoresistance. The non-malignant cells in the TME promote tumourigenesis throughout tumour development and metastasis.[6] The attention received by the ECM component of the TME has changed considerably over the last decade as its importance in tumour development, progression and metastasis was discovered. As of today, numerous articles can be found highlighting the tumour ECM and its role in malignancy and response to therapy.[4,5]

Tissue engineering is based on understanding tissue formation so new functional tissues can be developed in the laboratory.[7] The golden triad of tissue engineering is based on the combination of scaffolds, cells and molecular cues.[7] A scaffold is used by relevant cells to attach to, migrate through it, and proliferate to form the new tissue, and molecular cues are often needed to direct cell fate. These three components are instrumental in developing functional tissues and organs. Since these are also the basic components of cancer tissues, tissue engineers have endeavoured to produce tissue-engineered tumours in the laboratory in order to comprehend cancer mechanisms and test new therapies.[8–10] Tissue engineers possess an array of materials, namely polymers (both natural and synthetic), ceramics or a combination of them (composites), to develop degradable, functional and/or smart scaffolds to act as an ECM for recreating the TME.[9,10] However, the ECM is a complex entity in terms of both composition and structure that is constantly remodelled by tightly regulated remodelling mechanisms.[3,11] Therefore, many features of the ECM are lost when building scaffolds using the above-mentioned materials. As a result, tissue engineers often use decellularized matrices as scaffolds that offer the advantage of great similarity with the native tissue.[3,12] Decellularization is the process of eliminating native cells and genetic material from the ECM while ideally maintaining its structural, biochemical and biomechanical properties.[12] Decellularized tissues can then be repopulated with cells of interest.[12]

The vast majority of the research into tissue-engineered tumour models using decellularized tissues uses native tissue, and therefore a normal ECM, that is then repopulated with cancer cells and sometimes other non-cancerous cells associated with the tumour of interest.[3,13–15] However, the tumour ECM has unique features in terms of composition, for example, increased collagen deposition, and mechanical properties, that is, increased stiffness due to the heavy crosslinking of abundant collagen, resulting in a structurally and chemically different ECM to that of native tissues.[4,16] Therefore, very recently scientists started investigation into the decellularization of tumours, thereby producing decellularized tumour ECM (dT-ECM) that not only maintains the mechanical characteristics of the tumour but also retains its composition and structure. The use of these dT-ECM in tissue-engineered tumour models could very closely imitate actual tumours.

The aim of this paper is to review to date the efforts into decellularization of tumours, which in the authors' view represents a new frontier in the ever evolving field of tissue engineering and more specifically tumour tissue engineering. But first, the ECM and its important role in cancer will be described in order to frame the relevance and importance of decellularizing tumours to unravel key molecular and cellular mechanisms behind cancer.

The extracellular matrix and its importance in cancer

The extracellular matrix and its continuous remodelling

The non-cellular component found in all tissues and organs of our body is the ECM, of which two main forms exist: the interstitial ECM and the basement membrane.[5,11,17,18] The interstitial ECM is a porous and interconnecting three-dimensional (3D) network that surrounds stromal cells and can connect to the basement membrane, which is a stable, sheet-like dense structure that lines the basal surface of, for instance, epithelial and endothelial cells and separates tissues into different and well-organized compartments.[5] Apart from acting as a physical scaffold for cells, the ECM provides essential chemical, physical and mechanical signals necessary for tissue morphogenesis, differentiation and homoeostasis.[11,17] These various signals are detected by a multitude of cell surface receptors, thereby triggering intracellular signalling cascades that for instance result in the expression of relevant genes that regulate apoptosis, cell proliferation, migration or differentiation.[11,17] Throughout life in both healthy and diseased states like cancer, resident cell types secrete the molecules that make up the ECM, which are modified in response to mechanical cues and stimuli such as oxygen and nutrient availability.[11,19] Fundamentally, the ECM is composed of water, proteins and polysaccharides.[17] The major protein component is the collagen superfamily, which makes it the most abundant family of proteins in the human body.[20] The protein components of the interstitial ECM are mainly collagen types I, III, V, etc., fibronectin and elastin.[5,18] The basement membrane is mainly composed of collagen type IV, laminins and network-bridging proteins such as nidogen and heparan sulphate proteoglycans.[5,18] Nevertheless, each tissue and organ possess an ECM with a distinct composition and topology.[20]

Different remodelling mechanisms ensure the ECM is continuously changed in terms of structure, composition, abundance and organization of individual components.[5,21] Ultimately, changes in the ECM affect the microenvironment surrounding cells consequently changing cell behaviour. ECM remodelling is highly complex with over 700 proteins involved in the various mechanisms, which include: (1) ECM deposition, where the biochemical and mechanical properties of the ECM can be tuned by changing the amount and composition of ECM components; (2) chemical modification at the post-translational level, thereby altering the biochemical and structural characteristics of the ECM; (3) proteolytic degradation, where bioactive ECM fragments and ECM-bound factors are released; and (4) force-mediated physical remodelling, where the organization of the ECM is changed by aligning ECM fibres and creating new routes for cell migration.[5,21] Tissue homoeostasis depends on ECM remodelling and its tight regulation, as changing single elements of this delicate remodelling balance can have important effects on complex cellular signalling networks. This is because the various ECM components act as ligands for cell surface receptors such as integrins or tyrosine kinases.[22,23]

The extracellular matrix in cancer

Unsurprisingly, in pathological states like cancer, ECM remodelling is dysregulated.[5] Tumourigenic remodelling of the interstitial ECM in cancer induces a myriad of biochemical and biophysical changes that affect ECM stiffness, cell signalling and migration and tumour progression.[24] Furthermore, in malignant tumours, invasion of stromal tissue by cancer cells is a consequence of the tumourigenic remodelling of the basement membrane.[25] Cancer cells have the capacity to alter the four ECM remodelling mechanisms discussed in the previous paragraph.[5] As a result, a cancer-supporting ECM is assembled, which can comprise up to 60% of the tumour mass. The most notable features of tumour ECM are increased collagen deposition leading to an over-abundance of collagen, altered specific collagen types ratios and stiffness.[4,26]

The tumour ECM strongly differs in amount of deposition, composition, organization and post-translational modification from the ECM in normal tissue.[4] The tumourigenic ECM is deposited by CAFs as well as the tumour cells themselves.[27,28] Infiltration of fibroblasts and myofibroblasts and subsequent accumulation of significant amounts of a collagen-rich ECM is seen in many solid tumours. This process is called desmoplasia and is strongly linked to poor prognosis and resistance to systemic therapy.[29–31] CAFs contribute to a more malignant tumour phenotype by driving epithelial-to-mesenchymal transition (EMT) and induce production of collagen and other ECM molecules by supporting tumour cells via paracrine stromal cell-derived factor-1 (SDF1) and transforming growth factor beta (TGFβ) signals.[32–34] Accumulating evidence in the literature shows that platelets play a key role in promoting tumour growth and metastasis through interaction with tumour cells at multiple levels.[35,36] The ability of inducing platelet aggregation by many cells present in solid tumours correlates with metastatic potential.[35] Tumour cell-activated platelets secrete multiple factors that in turn target not only tumour cells but also others present in the TME thus affecting the tumour ECM.[35] Tumour-cell activated platelets crucially contribute to the establishment and expansion of the early metastatic niche and various mechanisms towards this effect have been proposed by researchers.[36] Briefly, the three most well-studied mechanisms are: (1) preparation of the initial metastatic microenvironment by forming the ECM and recruiting granulocytes, (2) creation of a neovasculature and (3) creation of an immune-suppressive environment around the developing metastases.[36]

Several ECM molecules such as fibrillar collagens, fibronectin, elastin and laminins are expressed in high levels in many solid tumours.[37,38] Some cancers are particularly rich in hyaluronan, for instance pancreatic ductal adenocarcinomas.[39] In comparison to benign mammary lesions, production of collagen is shifted towards collagen types I and III mainly in the stromal part of the tumour in invasive ductal carcinomas.[40,41] Since the benign lesions also consist mainly of fibroblastic cells, this manifests the tendency and capacity of breast tumour cells to change the secreted matrisome of its stroma. Furthermore, in the desmoplastic stroma of breast carcinomas, up to 15% of the collagen component of the matrix consists of collagen type V, which has low abundance in normal and fibrocystic ($<0.1\%$) breast tissue.[42] It has been shown that increasing the collagen V/I ratio reduces length and organization of collagen fibres to the point that fibre formation can be inhibited, resulting in a gel-like ECM.[43,44] Another example is ovarian carcinomas, where compared to benign tissue, collagen type IV is downregulated and its expression is inversely correlated with stage and markers of malignancy.[45] Increased collagen type I expression in melanoma is observed and corresponds with invasiveness, angiogenesis and reduced survival.[46,47] Finally, a fast collagen turnover in tumours is evidenced by high collagen mRNA expression, the aberrant form of fibrous collagen spindles and the increased expression of matrix proteases.[41]

The stiffness of the tumour ECM differs from normal ECM.[26,48] Tumour ECM stiffness is a consequence of tumour growth, and is related to an increase in metastasis rate and poor clinical outcomes.[49] Furthermore, tumour ECM stiffness disrupts tissue morphogenesis by increasing cell tension.[50] The increased stiffness in the tumour ECM results from an aggregation of ECM proteins that enclose packs of hyaluronic acid gel-like structures, which make tumours resistant to compressive stresses. In the

periphery, collagen and fibronectin are accumulated.[51] The increased collagen content and crosslinking are both correlated to an enhancement of ECM stiffness and cancer malignancy.[52] Regarding the increased degree of collagen crosslinking observed in tumour ECM, lysyl oxidase (LOX) is an amine oxidase responsible for collagen crosslinking and its amount is frequently elevated in cancers.[50] Increased matrix crosslinking forces tumour progression by providing enhanced integrin signalling. ECM stiffness may be involved in the rise of tumour incidence with ageing, as aged tissues are stiffer and contain high amounts of aberrant collagen crosslinking.[50]

As we have seen, the tumour ECM significantly differs from that in normal tissue: in a nutshell, the tumour ECM is more abundant, denser and stiffer. These altered features can negatively affect response to therapy.[4] For instance, an excessive accumulation of dense and rigid ECM, encapsulating clusters of tumour cells, can act as a barrier, thereby shielding the cells from therapeutic agents. Furthermore, this barrier also impairs diffusion of oxygen, nutrients and metabolites, leading to increased hypoxia and metabolic stress which activate anti-apoptotic and drug resistance pathways. Chemoresistance is also a consequence of cell–ECM contacts and increased tissue stiffness via integrin and FAK-signalling.[4]

This section merely offers an overview of the ECM and its alteration in cancer, which as the reader can gather, is a highly complex matter. If the reader wishes to learn more about the subject, there are numerous and excellent reviews which dwell on multiple or specific aspects and concepts discussed above.[4,5,16,26,35,36,51] Our aim was simply to highlight the importance of the ECM in cancer to set the tone for the following sections.

Decellularization of tumours

Rationale and importance

As mentioned in the Introduction, cancer is one of the biggest health challenges of our time, and tissue-engineered tumour models can help not only to comprehend cancer formation and progression mechanisms, but also to identify and test new therapies. The vast majority of tissue-engineered tumour models uses either a scaffold made of a biodegradable material, or decellularized healthy ECM, that are then populated with cancer cells. However, as seen in the previous section, the tumour ECM considerably differs from the ECM found in healthy tissues and therefore, models that use decellularized healthy ECM are missing important features of the ECM component of the TME. For instance, fibrillar collagens, fibronectin, elastin, and laminins are expressed in high levels in many solid tumours compared to the expression levels observed in non-tumoural tissues. Therefore, these expression levels will be missed in decellularized healthy ECM. Moreover, the increased levels of collagen, which as described in the previous section is heavily crosslinked, confer a stiffness to the tumoural ECM which will be absent from the decellularized healthy ECM. Consequently, producing dT-ECM for its use in tissue-engineered tumour models could very closely imitate actual tumours, which adds a level of mimicry absent from tissue-engineered models that use decellularized healthy ECM or fabricated scaffolds. We believe this added level of mimicry is needed to answer important questions about tumour formation and progression and identify new therapeutic targets.

A simple search on PubMed of ‘decellularized tumour’ rendered 251 results since 2010, out of which only 49 articles actually reported on dT-ECM (Figure 1). The rest of the articles were either reviews, or studies where decellularized healthy ECM was used to create tissue-engineered tumour models. As it can be seen, there is a positive trend in the number of publications that use decellularized materials for tissue-engineered tumour models. However, only a small fraction of them actually used decellularized tumours, for example, in the last year 2021, 50 publications reported on the use of decellularized materials for tissue-engineered tumour models, out of which 35 used healthy ECMs, 5 were reviews, and only 10 reported on dT- ECM. Since the tumour ECM is considerably different to native ECM, decellularized tumours could be a powerful tool in the study of cancer and new therapies for the reasons already exposed in this review. Before producing them, it is important to consider the tissue source, the decellularization method, the characterization of the dT-ECM and its recellularization with relevant cell types.

Tissue source

Tumour tissue can be obtained from different sources, namely human or animal derived, and cell culture derived. Table 1 shows the different options as well as their advantages and disadvantages.

Tumours isolated from the human body have a tumour ECM that is truthful to the real-life situation. On the other hand, ethical approval and patient consent can sometimes be tedious to obtain, which delays and constrains tissue collection for experimentation. Besides, tissue availability is limited when isolating tumours from human patients, which hampers large scale in vitro studies. Patient variability is also another disadvantage of using human-derived tumour ECM: even for the same cancer type, the ECM will vary between different patients.

Some of the disadvantages of using human-derived tissue can be solved with xenotransplantation models. In these models, human cells, tissues or organs can be implanted into an immunodeficient animal host, which provides the environment for the human tissue to function and grow normally.[71] These models have been studied for organ transplantation or toxicity studies,[71,72] and have

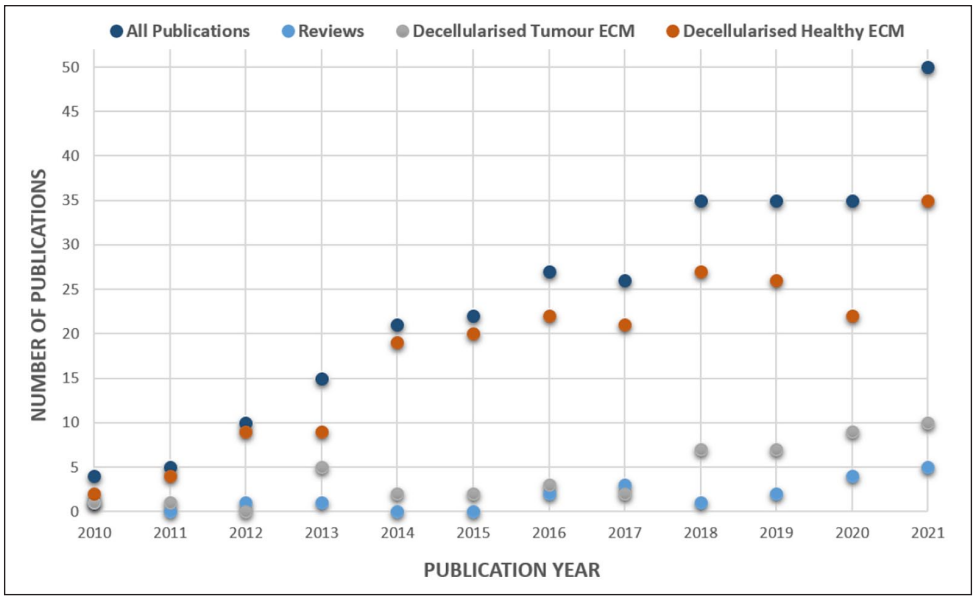

Figure 1. PubMed search for the phrase 'decellularized tumour' showing results from 2010 until 2021. Results are broken down into articles reporting into actual dT-ECM (decellularized tumour ECM), reviews, and the rest (studies where decellularized healthy ECM was used for tissue-engineered tumour models).

Table 1. Summary of tumour tissue sources discussed in the text.

Origin	Source	Advantages	Disadvantages
Human derived[53–61]	Tumours isolated from human patients	- Have a tumour ECM that is truthful to the real-life situation	- Procurement of ethical approval and patient consent - Tissue availability - Patient variability
	Tumours isolated from xenotransplantation models	- Have a tumour ECM that is truthful to the real-life situation - Eliminates issues of patient variability and tissue availability	- Procurement of ethical approval and patient consent - Need dedicated facilities for animal work - Animal work is costly
Animal derived[62,63]	Animal models of cancer	- Eliminates subject variability - Eliminates issue of tissue availability	- The tumour ECM will differ from that found in human tumours
	Induced	- Eliminates subject variability - Eliminates issue of tissue availability - Can isolate tumours at different stages of malignancy	- Procurement of ethical approval - Need dedicated facilities for animal work - Animal work is costly
Cell culture derived[64–70]	Cultured primary cancer cells Cultured cancer cell lines	- Eliminates issue of tissue availability - In the case of primary cancer cells, the deposited tumour ECM will resemble that found in patients in terms of composition - In the case of cancer cell lines, subject variability is eliminated - Cost-effective	- The tumour ECM will differ from that found in human tumours - The tumour ECM can be easily altered by the culture conditions - Procurement of ethical approval to use primary cancer cells

emerged as a powerful research tool in the fight against cancer.[53,54] With these models, it is possible to grow an unlimited number of patient-specific tumours that can then be harvested for experimentation, including decellularization studies. Alternatively, cancer cell lines can also be used to induce the tumours.[54–58] For instance, Wishart and colleagues[55] used the epithelial, human breast cancer cell line MDA-MB-231 to create a xenotransplantation model of breast cancer. MDA-MB-231 cells were injected into the mammary gland of immunocompromised mice and were left to grow for 8 weeks. Parallel to this, PyMT-MMT mice (mammary tumour transgenic mouse model carrying the polyomavirus middle T antigen oncogene) were kept for 10 weeks, as this is a mouse model of breast cancer that

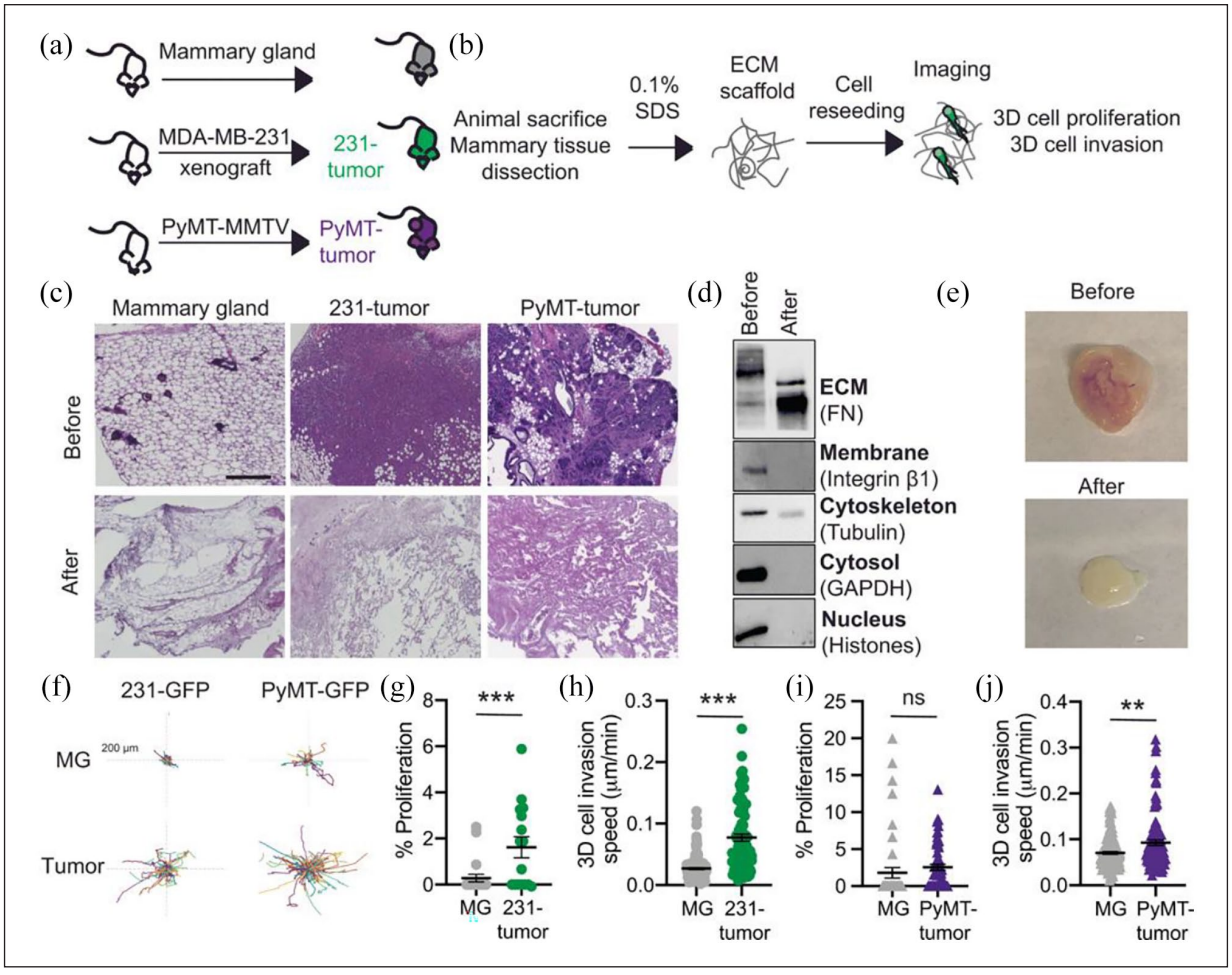

Figure 2. (a) Generation of tumours with MDA-MB-231 xenografts (231-tumour) and tumours from PyMT-MMTV (PyMT-tumour) mice. (b) Study design. (c) Haematoxylin and eosin (H&E) staining of mammary gland, 231-tumours and PyMT-tumours before and after decellularization (scale bar = 200 µm). (d) Representative western blot of 231-tumour before and after decellularization for cellular compartments. (e) Photo before and after decellularization of PyMT-tumour. (f) Representative tracks (each line is a cell over 16 h) of 231-GFP or PyMT-GFP cells seeded on decellularized ECM from mammary gland (Mg), 231-tumours or PyMT-tumour. (g) Percentage of proliferation for 231-GFP cells on healthy mammary gland or 231-tumour. (h) Cell migration speed of 231-GFP seeded on decellularized ECM from mammary gland and 231-tumours. (i) Percentage of proliferation for PyMT-GFP cells on mammary gland or PyMT-tumour scaffolds. (j) Cell migration speed of PyMT-GFP seeded on decellularized ECM from mammary gland and PyMT-tumours. For proliferation, data point is a field of view. For cell migration, each point is the average speed of a cell over 16 h. Data shows mean ± SEM from at least three decellularized ECM scaffolds from three mice. Statistical significance was analysed by unpaired two-tailed Mann Whitney t test. $^{**}p < 0.01$ and $^{***}p < 0.005$. ns, not significant. Image from Wishart et al. 2020[55] (open-access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited).

grows tumours in all mammary glands that closely resemble the progression and morphology of human breast cancers.[62] The authors reported that at the 8 and 10 weeks' time points all tumours had metastases to the lungs. The tumours were extracted and successfully decellularized using a detergent-based protocol (Figure 2(a)–(e)). They were then seeded with MDA-MB-231 cells that had been labelled with green fluorescent protein (GFP) and live imaged for 16 h, showing that ECM isolated from xenograft 231-tumours significantly increased cell migration and proliferation (Figure 2(f)–(h)). Seeded GFP-labelled PyMT tumour–derived cells in the ECM isolated from primary MMTV-PyMT tumours increased tumour cell invasion without affecting proliferation (Figure 2(f)–(j)). The authors concluded that whole-tissue ECM can be isolated from healthy and tumour-containing mammary glands and used to study ECM-driven effects on individual cell phenotypes.[55]

Animal derived tumour tissue is also a relevant source for decellularization procedures. Animal models of cancer exist, like the just mentioned PyMT-MMT mice model of breast cancer.[62] The option of inducing a tumour is also

possible. For example, Saldin and colleagues[63] induced oesophageal adenocarcinoma in Sprague-Dawley rats by subjecting them to the Levrat surgical procedure. In this procedure, the jejunum is anastomosed to the distal oesophagus, thereby creating constant acid reflux and chronic inflammation in the distal oesophagus. Over the next 17–33 weeks, normal oesophageal squamous epithelium transforms to metaplastic Barrett's oesophagus (i.e. intestinal metaplasia) and then to a neoplastic, glandular cell type. These changes in cell phenotype mimic the pathophysiology of gastroesophageal reflux disease. The advantage of this procedure is that the authors could harvest the tumour tissue at different stages of the disease.[63] Some of the disadvantages of using tumour tissue isolated from human patients, for example, patient-specificity and tissue availability, are solved by using animal derived tumour tissue as a source. Nevertheless, ethical constraints are still a disadvantage of this tumour tissue source. Furthermore, dedicated facilities for animal work are needed, which are not always easily accessible. This is also the case with xenotransplantation models.

In cell culture derived tumours, cells (primary or cancer cell lines) are cultured using standard mammalian tissue culture techniques, and the tumour ECM deposited by the cells over time is then decellularized and used for investigation.[64–68] Cell culture derived tumour ECM offers evident advantages such as plenty availability and cost-effectiveness. Moreover, in the case of primary cancer cells, the deposited tumour ECM will resemble that found in patients in terms of composition, whilst in the case of cancer cell lines, subject variability is eliminated. Nevertheless, the deposited ECM will differ from that found in real tumours, especially in the case of tumour ECM derived from cultured cell cancer lines. However, this is an important source of tumour ECM for experimentation as evidenced by the literature. For instance, Wang and colleagues[69] investigated the underlying mechanisms behind the acquisition of resistance to EGFR (epidermal growth factor receptor) tyrosine kinase inhibitors (TKIs), which remains a critical problem in lung cancer. Although it is known that the TKI-induced or -selected genetic changes drive resistance, this also occurs without genetic changes in tumour cells. The authors showed that the ECM derived from various components of the TME may drive resistance in the absence of genetic changes: culturing tumour cells that were otherwise sensitive to EGFR TKIs on dT-ECM, or co-culturing with the tumour ECM donor cells, immediately conferred resistance to them. Furthermore, they showed that the primary constituent in the tumour ECM driving resistance may be collagen, partly through the collagen receptor Integrin-β1. The effect of the tumour ECM and collagen was dose-dependent and reversible, thus revealing a potential therapeutic target. The dT-ECM used to reach these relevant conclusions was derived from the culture of lung cancer cell lines and fibroblasts.[69] A last and interesting example is the work by Nayak and colleagues.[70] In this study, the authors engineered a biochemico- and mechano-mimetic 3D culture platform for primary breast cancer cells by decellularizing CAFs cultured on 3D macroporous polycaprolactone (PCL). Briefly, the authors cultured CAFs for 7 days on 3D porous PCL scaffolds that had an elastic modulus (4.0 ± 0.5 kPa) comparable to that of breast tumours (4.04 ± 0.93 kPa). Decellularization included various methods such as freeze-thaw and detergents. As a result, the decellularized scaffolds had a PCL backbone, which mimicked the mechanical properties of breast tumours, coated with a natural tumour ECM, which mimicked the biochemical properties of metastatic breast tissue.[70] Other studies beyond decellularization have also developed tissue-engineered tumour models based on the culture of cancer cell lines, like the work by Martinez and co-workers where the luciferase-tagged SKOV-3 ovarian cancer cell line, the CAFs cell line human ovarian cancer fibroblast IHFOT-208 and the OVCAR-8 cell line were used to create a preclinical 3D ovarian cancer model using a perfusion bioreactor.[73] A last example is the paper by Stamati and colleagues[74] were CAKI-2 and 786-O RCC renal cell carcinoma lines were used to create biomimetic 3D renal tumoroids to study the effect of the tyrosine kinase inhibitor Pazopanib on killing cancer cells and disruption of endothelial networks.

Decellularization methods, characterization and recellularization

As of today, the same methods used in the decellularization of tissues and organs are used to decellularize tumours.[3,12] These include biological, chemical or physical methods.[12,75] Within each method category, different agents and techniques exist. Usually, a combination of methods is used (Table 2).

Biological decellularization methods include the use of enzymes to break down DNA or RNA fragments (nucleases), cell-matrix interactions (trypsin) or target peptide bonds (pepsin). They are normally used with other chemical agents like surfactants, which disarrange the phospholipid cell membrane. For instance, D'Angelo and colleagues[77] used a surfactant-enzymatic treatment on both healthy liver and colorectal metastatic liver and reported a phenotypical appearance similar to the non-decellularized tumours. Other chemical methods like hypertonic and hypotonic solutions, which induce an osmotic shock, have also been used in the decellularization of tumours alongside other methods and agents. As an example, Pinto and co-workers[78] used a decellularization method on colorectal specimens, both tumour and normal, that included a first step using a hypotonic buffer, followed by a 24 h treatment with 0.1% SDS, three washes with the hypotonic buffer, and a

Table 2. Summary of decellularization methods used to decellularize tissues including tumours.

Method	Agent (examples)/technique	Description	Comments
Biological[3,12,75–78]	Enzymes (nucleases, trypsin, pepsin)	- Nucleases break down DNA or RNA fragments. - Trypsin breaks down cell-matrix adhesions. - Pepsin targets peptide bonds.	- Ineffective on their own so they are used in combination with other methods and agents.
Chemical[3,12,75–78]	Surfactants (sodium dodecyl sulphate (SDS), sodium deoxycholate (SD), Triton X-100, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulphonate (CHAPS))	- Surfactants disarrange the phospholipid cell membrane, thereby lysing cells. - They can be ionic, non-ionic or zwitterionic.	- SDS is effective at removing cells and genetic material but it is cytotoxic, thus it needs extensive washing. - SD is less damaging and cytotoxic than SDS. - Non-ionic surfactants like Triton X-100 are less damaging to tissue structure than ionic surfactants like SDS and SD. - They can cause DNA agglutination. - Surfactants tend to be used in combination with other methods and agents.
	Acids and bases (alkaline treatment, peracetic acid, organic acids)	- Acids and bases solubilize the cell membrane and nuclear material due to their intrinsic charge properties.	- Peracetic acid is highly corrosive and has a strongly oxidising nature. - Peracetic acid increases ECM stiffness. - Used in combination with other methods and agents.
	Hypotonic and hypertonic solutions	- These solutions use osmotic properties to make the cells explode.	- The osmotic shock kills the cells, but it does not remove the cell waste that it releases to the matrix.
Physical[3,12,75,76,79–88]	Agitation/stirring/rotation/ shaking	- Commonly used to facilitate chemical agent infiltration and to induce cell lysis.	- As a facilitator, it needs to be used in combination with other methods. - An aggressive agitation or rotation can greatly damage the ECM.
	Freeze-thaw	- Freezing temperatures are alternated with biological temperatures for an optimized number of cycles, thereby lysing cells.	- Ineffective at removing cells and genetic material. - It is used in combination with other methods and agents.
	High hydrostatic pressure	- Applying pressures above 600 MPa to dismantle the cellular membrane.	- It can be used on its own. - Excessive pressure can damage the structure.
	Supercritical CO_2	- Supercritical fluids have liquid-like density and gas-like diffusivity. - With a critical temperature of 31.1 °C and a critical pressure of 7.40 MPa, supercritical CO_2 is compatible with biological systems.	- CO_2 does not remain within the tissue and therefore, extensive washing is not required. - CO_2 is non-polar, so a polar entrainer is necessary to remove the polar phospholipid membrane. - It can be used on its own.
	Sonication	- Sonication is the process of applying sound energy to agitate samples in a liquid. - Ultrasonic frequencies ($>$20 kHz) are usually used. - Enables chemical agents to reach the more inner parts of the tissue.	- As a facilitator, it needs to be used in combination with other methods. - An aggressive sonication process can greatly damage the ECM.
	Vacuum-assisted decellularization	- Enables chemical agents to reach the more inner parts of the tissue.	- As a facilitator, it needs to be used in combination with other methods.

Table 3. Summary of common methods used in the characterization of decellularized ECM, either obtained from healthy tissue or tumours.

Purpose	Characterization method	Comments
Elimination of cellular material	H&E staining	Stains cell nuclei purple/blue and the ECM pink
	DAPI or Hoechst stainings	Bind to AT-rich regions of dsDNA
	Masson's trichrome staining	Stains cell cytoplasm red and collagens blue
	Quantification of DNA content	Quantifies the amount of DNA present in the ECM
Preservation of the original ECM	Immunohistochemical staining of specific ECM components, e.g. laminin, fibronectin, collagens type I and IV	Detects the pattern of expression of specific ECM components
	Alcian blue or Toluidine blue stainings	Stain glycosaminoglycans (GAGs) blue
	Periodic acid-Schiff staining	Detects polysaccharides like glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues
	Silver stain	Detects reticular collagen fibres
	Quantification of collagen content	Quantifies the total amount of collagen present in the ECM
	Quantification of GAGs content	Quantifies the total amount of GAGs present in the ECM
	Proteomics	Characterizes the proteome, that is, the entire set of proteins present in the ECM.
	Fourier transform infrared spectroscopy (FTIR)	Characterizes the infrared spectrum of the ECM
	Scanning electron microscopy (SEM)	Characterizes the architectural features of the ECM, e.g. pores, fibres
	Quantification of architectural parameters with an image analysis software	Characterizes percentage of porosity, pore size range or fibre diameter
	Mechanical testing	Characterizes mechanical properties of interest, which include tensile strength, elastic modulus, stiffness or yield strength
	Rheology	Characterizes the viscoelastic properties of the ECM

Table compiled from references.[12,55,56,61,75–78,89]

3 h digestion with DNase. The authors performed the protocol under constant agitation to aid the infiltration of the different agents into the samples. It was reported that no visible nuclei could be observed in normal and tumour matrices after decellularization. DNA quantification confirmed the efficient removal of about 99.3% of the total DNA in normal tissue and 99.6% in tumours. Haematoxylin and Eosin (H&E) and Masson's trichrome staining confirmed the absence of cell remnants in both normal and tumour decellularized tissues with preservation of a collagen-rich ECM network.[78] The study by Pinto et al. evidences that some physical methods like agitation, rotation, shaking or sonication can facilitate the decellularization process.[83–86] Freeze-thaw is another physical method that has been used in the decellularization of tumours. The work by Romero-López and colleagues[87] is an example, where an initial freeze-thaw cycle was included in the decellularization protocol, followed by surfactant treatment and extensive washing to eliminate the detergents. The process was aided by the use of stirring. Other promising and efficient physical methods such as high hydrostatic pressure or supercritical CO_2, which yield excellent results in normal tissues,[79–82,88] have not yet been used in the decellularization of tumours to the best of the authors' knowledge.

Characterization of the dT-ECM follows the decellularization process. As with decellularized healthy tissues, the main objective is to confirm elimination of cellular material and preservation of the original ECM, including composition, structure and mechanical properties.[12] Table 3 summarizes available methods commonly used to achieve this objective. Typically, the native or non-decellularized tissue is characterized alongside the decellularized one for comparison and assessment of efficiency.

Macroscopically, the decellularization process can be followed by observing the change in colour and light transmittance from the native tissue (pink to orange/reddish and opaque) to the decellularized one (white and translucent) (Figure 3). Histological and immunohistological stainings are popular and somewhat unavoidable methods for assessing decellularization. Not only they detect specific components and structures but also some of them can qualitatively confirm elimination of cellular material, that is, H&E and Masson's trichrome stainings. DAPI (4′,6-diamidin-2-fenilindolo) or Hoechst (bis-benzimides) stainings can also be used to qualitatively confirm elimination of cellular material as they bind to AT-rich regions of dsDNA (double stranded DNA). Nevertheless, quantitative confirmation can be obtained by quantifying the DNA content. As an example the work by Lü and colleagues,[56] where

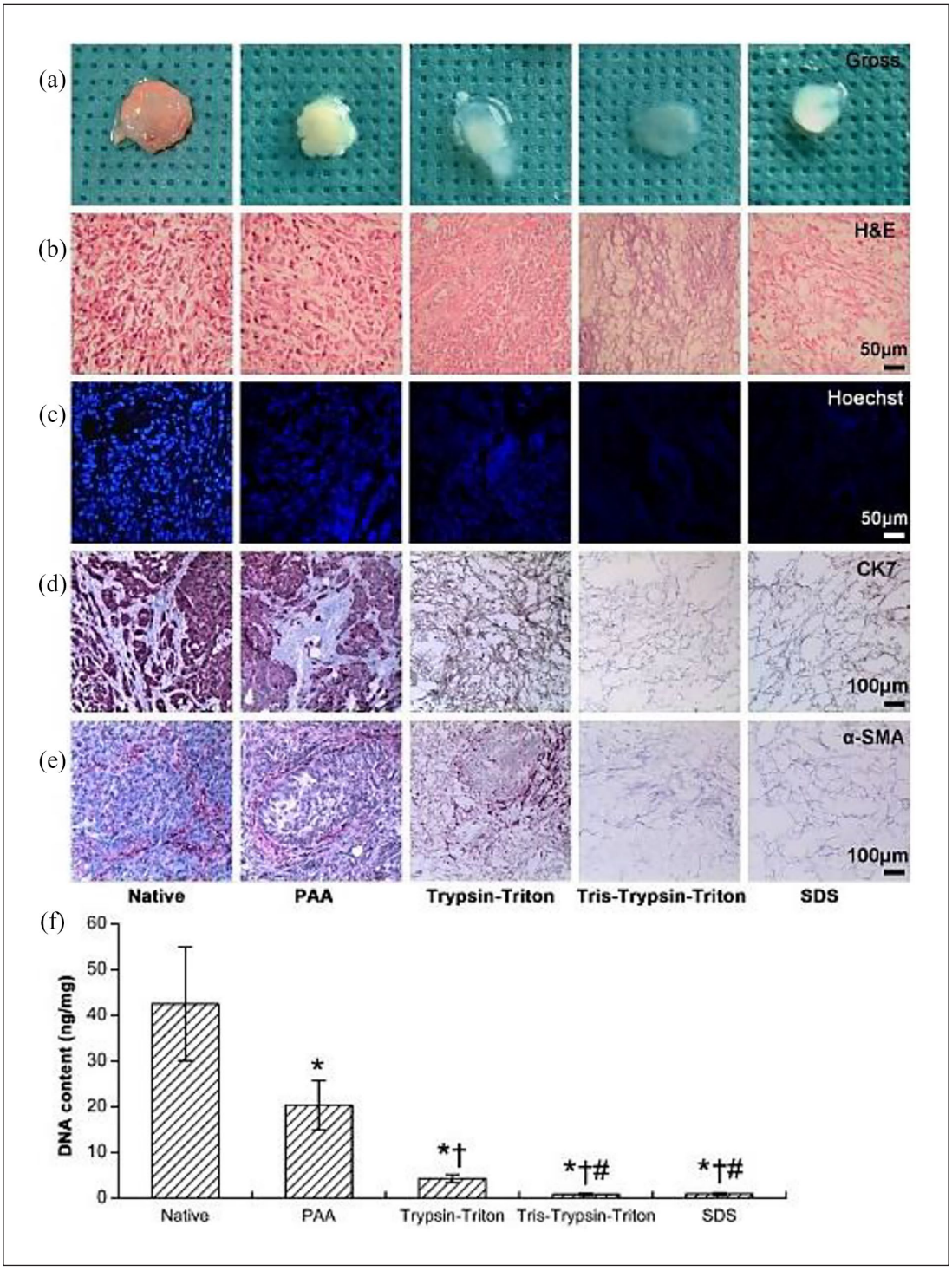

Figure 3. Example of cellular removal evaluation in the decellularization of solid tumours derived from A549 human pulmonary adenocarcinoma cell xenotransplantation using different procedures: peroxyacetic acid (PAA), Trypsin-Triton, Tris-Trypsin-Triton and SDS. (a) Gross appearance showing change in colour and light transmittance. (b) H&E and (c) Hoechst stainings showing complete cellular removal for some of the decellularization treatments (i.e. Tris-Trypsin-Triton and SDS). (d) Immunohistochemical staining for CK7 and (e) α-SMA showing complete removal of epithelial and mesenchymal cell components for Tris-Trypsin-Triton treated or SDS treatments. (f) DNA content quantification showing mean ± SD ($n = 10$, $^*p < 0.05$ versus the native group). Statistical significance was analysed between multiple groups using the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. A value of $p < 0.05$ was considered significant ($\dagger p < 0.05$ versus the PAA group; $\#p < 0.05$ versus the Trypsin-Triton group). Image from Lü et al. 2014[56] (open-access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited).

tumours produced from human pulmonary adenocarcinoma A549 cells subcutaneously injected in SCID mice were decellularized with four different procedures that combined chemical and biological methods. Their analysis of cellular removal included H&E and Hoechst stainings, immunohistochemical staining for cytokeratin 7 (CK7) and alpha smooth muscle actin (αSMA), and quantification of DNA content. Combination of the results rendered the SDS-based decellularization protocol as the most efficient (Figure 3).[56] Finally, confirmation of preservation of the original ECM entails compositional, architectural and mechanical analyses. Information about the ECM composition can be obtained with immunohistochemical and histological stainings, by quantifying the total amount of collagen or GAGs, by proteomic analysis, which characterizes the entire set of proteins present in the ECM (proteome), or by Fourier transform infrared spectroscopy (FTIR). Popular methods for architectural or structural analysis include scanning electron microscopy (SEM), whilst mechanical testing or rheology can give information about tensile strength, elastic modulus, stiffness, yield strength or viscoelasticity. In the previously cited work by Pinto et al.[78] on decellularization of colorectal specimens, both tumour and normal, rheology was used to evaluate the impact of decellularization on the viscoelastic properties of normal and tumour matrices. The complex shear modulus (G*) data was retrieved from the linear viscoelastic region (LVR) of the frequency sweeps. Results showed that tumour native tissues were stiffer than the normal ones and that these differences were preserved after decellularization in all cases.[78]

When building tissue-engineered tumour models using dT-ECM, recellularization with relevant cell types follows the decellularization process after its successful characterization. Ideally, to create a truly identical tumour model, all the cells present in the TME should be introduced into the dT-ECM to recapitulate the cellular complexity of live tumours. However, this is an intricate and complicated task. Therefore, researchers tend to focus on one or two cell types to slowly unravel their behaviour in the TME.[59,63,84] Technically, as with other decellularized materials, recellularization of dT-ECM can be accomplished by simply seeding the cell suspension onto the dT-ECM piece.[91,92] Culture of the seeded dT-ECM can be done statically or dynamically[90,91] and special attention should be paid to seeding efficiency, that is, percentage of seeded cells that actually attach to the dT-ECM scaffold, that may be optimized as done in other tissue engineering applications.[92]

Examples of research using decellularized tumours

Despite the limited number of studies reported in the literature, research using decellularized tumours has already produced important results, like indication of potential therapeutic targets,[87,93] unravelling of malignancy mechanisms,[59,63,77,83,84,94,95] understanding the regulation of angiogenesis in tumours[67,96] or response to chemotherapy agents.[69] Some specific examples are discussed next where the relevance of using dT-ECM instead of healthy ECM easily transpires. Furthermore, the examples also clearly show that the dT-ECM retains tissue-specificity as they can support normal cancer cells (as well as other cells of the TME) behaviour.[59,63,84,87]

Intrahepatic cholangiocarcinoma (iCCA) is a highly lethal liver cancer that arises from the intrahepatic biliary tree. This malignancy is the second most common after hepatocellular carcinoma, and accounts for 10% of all primary liver malignant neoplasms.[93,97] Since its incidence and mortality are increasing worldwide, it represents a significant health problem. Late and silent clinical manifestations, high recurrence rate after surgical resection, and limitations in treatment options lead to very high levels of mortality. Carpino and colleagues[93] performed matrisome analysis of the ECM of iCCA to investigate the importance of the ECM proteins in the pathogenesis of iCCA. They used surgically resected iCCA tissues as well as surrounding non-cancerous tissues that were decellularized using an SDS-based protocol. Their results showed that iCCA ECM had high levels of collagen fibres and low abundance of reticular and elastic fibres, which suggested increased stiffness and loss of polarity. When compared to the ECM obtained from the surrounding noncancerous tissues, the basement membrane of iCCA ECM was dismantled, angiogenesis was reduced, and oncosuppressive activity was downregulated. The authors focussed on the effects of the overexpression of collagen type III alpha 1 chain (COL3A1) in iCCA, and provided evidence that COL3A1 promotes iCCA cells migration, as well as being a component of tumour-associated aligned collagen. The authors concluded by indicating collagen type III as a therapeutic target for the treatment of iCCA.[93] Based on these results, it would be advisable to investigate the feasibility of collagen type III as a therapeutic target in iCCA, followed by the design of a system to deliver the therapeutic agent onto the desired site, as collagen type III is one of the important components of the liver ECM and changes in its relative composition can lead to liver pathologies like cirrhosis.[98]

Oesophageal adenocarcinoma is a sub-type of oesophageal carcinoma that affects the glandular cells of the lower oesophagus at the junction with the stomach. It results from the chronic exposure of the squamous epithelium to gastric contents and thus, it is a chronic inflammatory-driven cancer. However, there is still limited understanding of the changes suffered by the ECM during disease progression. Therefore, the aim of Saldin and co-workers[63] was to investigate the effect of ECM harvested from normal, metaplastic, and neoplastic oesophageal tissue upon the activation state of macrophages, which are cells of interest in therapeutic applications. Normal, metaplastic and neoplastic ECM nanofibrous hydrogels were prepared from decellularized oesophageal adenocarcinoma tissue

isolated from rats that were subjected to the Levrat surgical procedure to induce oesophageal adenocarcinoma as described earlier in this review. Results showed that in the neoplastic oesophageal ECM, proteins functionally related to cancer and tumourigenesis were identified, including collagen alpha-1(VIII) chain (COL8A1), lumican and elastin. Metaplastic and neoplastic ECM induced distinctive effects upon THP-1 macrophage signalling compared to ECM from normal oesophageal tissue. These effects included activation of pro-inflammatory IFNγ and TNFα gene expression and anti-inflammatory IL1RN gene expression. Furthermore, neoplastic ECM robustly increased macrophage TNFα protein expression. The authors also showed that the secretome of macrophages pre-treated with metaplastic and neoplastic ECM increased the migration of normal oesophageal epithelial cells, which also occurs with tumour cells. In summary, results suggested the abnormal signals also exist within the pre-cancerous state.[63] Therefore, this information could be used for early detection of the pathology, which would mean less aggressive and more successful treatment of oesophageal adenocarcinoma.

Another interesting example is the work by Landberg and colleagues[59] who developed an experimental platform using cell-free patient-derived scaffolds (PDSs) from primary breast cancers that were infiltrated with standardized breast cancer cell lines, MDA-MB-231 and MCF7 specifically. The authors observed that the developed models induced a series of orchestrated changes in differentiation, EMT, stemness and proliferation of the cancer cell population. Additionally, an increased cancer stem cell pool was seen and global gene expression profiling showed that PDS cultures were similar to xenograft created in mice using the same breast cancer cell lines. Cell-free PDSs were analysed by mass spectrometry revealing subgroups based on protein composition that were linked to clinical properties such as tumour grade. An induction of EMT-related genes in the cancer cells grown on the PDSs were significantly associated with clinical disease recurrences in breast cancer patients.[59] Therefore, the PDSs mimicked *in vivo*-like growth conditions and would be a powerful tool to uncover the malignancy-inducing properties of the TME. This study is a clear example of why using dT-ECM can offer the possibility of studying the role of the unique tumour ECMs that appear during cancer progression in malignancy mechanisms. A system like the one developed by Ladberg could be used to find therapeutic targets for all stages of breast cancer.

Yet another example of the importance of using dT-ECM is the study by Genovese et al. where in order to investigate the ECM role in the modulation of tissue homoeostasis and tumourigenesis, ECM from mucosae from healthy human colon, perilesional area, and colorectal carcinoma was obtained from patients undergoing colorectal carcinoma resection surgery.[84] The samples were decellularized using common methods already discussed in this review, that is, hypotonic and hypertonic solutions, non-ionic detergents and enzymes. Interestingly, the decellularized ECM showed different 3D structures between normal, perilesional and tumour-derived stroma. Moreover, the localization and organization of seeded monocytes and cancer cells was differentially regulated by the three types of ECM. Furthermore, healthy, but not perilesional and colorectal carcinoma-derived ECM, constrained cancer cells invasion. Cell proliferation was highest in tumour derived-ECM, followed by perilesional, and healthy-derived ECMs.[84] These types of decellularized scaffolds produced by Genovese and colleagues could be very useful for finding therapeutic targets for antineoplastic drugs, by targetting ECM components present in perilesional and tumoural regions.

Finally, Romero-López and colleagues demonstrated that the tumour ECM has a central role in cancer cell growth and associated vasculature by using decellularized ECM from colon tumour that had metastasized to liver and normal human colon.[87] The decellularized ECM was reconstituted into hydrogels that were seeded with endothelial colony forming cells or cancer cells. The authors reported important differences in protein composition, stiffness, vascular network formation and tumour growth between the normal and the tumour ECM. Furthermore, cells seeded in dT-ECM had a higher glycolytic rate than those seeded in normal ECM. The authors concluded by suggesting that targetting the tumour ECM may be a potential adjunct anti-tumour therapy.[87] It would be recommended to keep researching the tumour ECM as a whole or some of its individual components as therapeutic targets, focusing on how to deliver these therapeutics to the tumour site without altering the surrounding tissue ECM, as many of the components present in the tumoural ECM are also part of the healthy ECM, as already seen in this review.

Concluding remarks and future direction

Cancer is a devastating disease and one of the leading causes of death worldwide. In recent years, scientists are regarding cancer as distinct tissues or organ-like structures, to better understand the mechanisms of cancer cells behaviour and progression. The various cancer tissues have an ECM that plays a key role in regulating cell behaviour as well as metastasis. Given the uniqueness of the tumour ECM, which differs in terms of composition and structure from that found in healthy tissues, decellularized tumours can be powerful tools in understanding cancer formation and progression and the search of therapeutic targets to treat it. This review summarizes the state-of-the-art to date in terms of the various tumour sources, decellularization methods, characterization and recellularization techniques, and scientific achievements of research using dT-ECM.

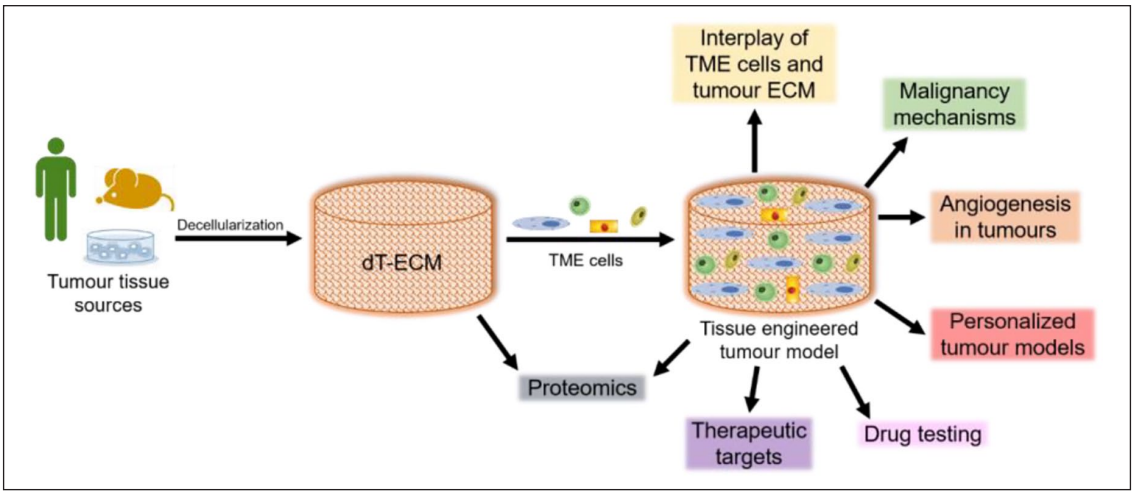

Figure 4. Conceptual diagram showing the main points discussed in this review. Tumours can be obtained from various tissue sources, namely human, animal or cell culture-derived. They can then be decellularized to obtain dT-ECM which possess the distinct composition, architecture and mechanical properties of the tumour ECM. In order to create tissue engineered tumour models, the dT-ECM can be repopulated with cells found in the TME. These models could be cultured using a variety of culture systems and used to study the interplay between TME cells and the tumour ECM, malignancy and angiogenesis mechanisms, proteomics, novel therapeutic targets, effect of anti-cancer drugs, and to create personalized tumour models for precision medicine. Proteomic analysis could also be performed on unseeded dT-ECM.

In terms of sources, it is worth remarking that human-derived and primary cell culture-derived ones offer the possibility of creating patient-specific tumour models for testing personalized therapeutic regimes. As medicine moves from a one-size-fits-all approach towards personalized or precision medicine, this is an important feature to take into account. So far, the same decellularization methods used for decellularizing healthy tissue ECM are used to produce dT-ECM, especially biological and chemical methods.[3,12] Nevertheless, as already mentioned, some physical methods such as high hydrostatic pressure or supercritical CO_2, which produce excellent results in normal tissues, have yet to be used in the decellularization of tumours. Therefore, this could be an area for further investigation. Similarly, more investigation into the mechanical properties of dT-ECM is recommended as characterization tends to focus on confirming removal of cellular material and retention of the original composition. Since stiffness is one of the distinct features of the tumoural ECM, assessing the mechanical properties of dT-ECM should be of prime importance.

Regarding the tumour models that can be built using dT-ECM (Figure 4), there are promising culture systems like microfluidic devices that have to be yet used.[99–101] The miniaturization potential as well as dynamic culture conditions that offer these devices could be very useful for producing mini-models and culturing them under physiological flow conditions. There are examples in the literature of tumour-on-a-chip models however, to the best of our knowledge, none of them include dT-ECM.[99,101] Moreover, the built models should incorporate as many cells from the TME as possible, including cancer stem cells,[102] as they are all important in the tumorigenic process. Therefore, we believe that fabrication of dT-ECM-based tumour models should go towards increasing complexity in terms of incorporating cellular components from the TME, as well as miniaturization and physiological flow culture conditions. Furthermore, detailed ECM compositions in various tumour contexts are still unclear, as are the specific targets of different proteases. Optimized proteomics analysis now provides tools to study the entire tumour ECM. Unseeded dT-ECM at various stages of malignancy could be used to elucidate these compositions. Different cancer stages could also be produced by seeding TME cells on dT-ECM, allow dT-ECM remodelling before proceeding to carry out proteomic analysis (Figure 4).

In conclusion, important efforts have been made, but this review highlights that more research is needed and we have indicated areas for future investigation. Research into dT-ECM and its use in cancer models has the potential of answering important questions related to various aspects of tumour formation and progression. Ultimately, this research can identify new and effective therapeutic targets and, within the near-future of personalized medicine, create patient-specific tumour models and therapeutic regimes.

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References

1. International Agency for Research on Cancer. *World Health Organization*. https://gco.iarc.fr/ (2020).
2. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144:646–674.
3. Hoshiba T. Decellularized extracellular matrix for cancer research. *Materials* 2019; 12:1311.
4. Henke E, Nandigama R and Ergün S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. *Front Mol Biosci* 2019; 6:160.
5. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, et al. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun* 2020; 11:5120.
6. Baghban R, Roshangar L, Jahanban-Esfahlan R, et al. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Commun Signal* 2020; 18:59.
7. Langer R and Vacanti JP. Tissue engineering. *Science (80-.)* 1993; 260:920–926.
8. Ricci C, Moroni L, Danti S. Cancer tissue engineering? new perspectives in understanding the biology of solid tumours? a critical review. *OA Tissue Eng* 2013; 1(1): 4. https://www.oapublishinglondon.com/article/60
9. Sitarski AM, Fairfield H, Falank C, et al. 3D tissue engineered in vitro models of cancer in bone. *ACS Biomater Sci Eng* 2018; 4(2): 324–336.
10. Kar S, Molla MS, Katti DR, et al. Tissue-engineered nanoclay-based 3D in vitro breast cancer model for studying breast cancer metastasis to bone. *J Regen Med Tissue Eng* 2019; 13:119–130.
11. Yue B. Biology of the extracellular matrix: an overview. *J Glaucoma* 2014; 23:S20–S23.
12. García-Gareta E, Abduldaiem Y, Sawadkar P, et al. Decellularised scaffolds: just a framework? Current knowledge and future directions. *J Tissue Eng* 2020; 11:2041731420942903.
13. Brancato V, Ventre M, Imparato G, et al. A straightforward method to produce decellularized dermis-based matrices for tumour cell cultures. *J Regen Med Tissue Eng* 2018; 12:e71–e81.
14. Yi H-G, Jeong YH, Kim Y, et al. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. *Nat Biomed Eng* 2019; 3:509–519.
15. Keeton SJ, Delalande JM, Cranfield M, et al. Compressed collagen and decellularized tissue – novel components in a pipeline approach for the study of cancer metastasis. *BMC Cancer* 2018; 18:622.
16. Nallanthighal S, Heiserman JP and Cheon DJ. The role of the extracellular matrix in cancer stemness. *Front Cell Dev Biol* 2019; 7:86.
17. Frantz C, Stewart KM and Weaver VM. The extracellular matrix at a glance. *J Cell Sci* 2010; 123:4195–4200.
18. Kular JK, Basu S and Sharma RI. The extracellular matrix: structure, composition, age-related differences, tools for analysis and applications for tissue engineering. *J Tissue Eng* 2014; 5. https://journals.sagepub.com/doi/10.1177/2041731414557112?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed
19. Bhattacharjee O, Ayyangar U, Kurbet AS, et al. Unraveling the ECM-immune cell crosstalk in skin diseases. *Front Cell Dev Biol* 2019; 7:68.
20. García-Gareta E. *Collagen, from tissue culture to biomaterials, tissue engineering, and beyond*. Newcastle: Cambridge Scholars Publishing, 2019.
21. Bonnans C, Chou J and Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014; 15:786–801.
22. Barczyk M, Carracedo S and Gullberg D. Integrins. *Cell Tissue Res* 2010; 339:269–280.
23. Paul MK and Mukhopadhyay AK. Tyrosine kinase - role and significance in cancer. *Int J Med Sci* 2004; 1:101–115.
24. Egeblad M, Rasch MG and Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. *Curr Opin Cell Biol* 2010; 22:697–706.
25. Kessenbrock K, Plaks V and Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; 141:52–67.
26. Najafi M, Farhood B and Mortezaee K. Extracellular matrix (ECM) stiffness and degradation as cancer drivers. *J Cell Biochem* 2019; 120:2782–2790.
27. Casey T, Bond J, Tighe S, et al. Molecular signatures suggest a major role for stromal cells in development of invasive breast cancer. *Breast Cancer Res Treat* 2009; 114:47–62.
28. Naba A, Clauser KR, Hoersch S, et al. The matrisome: in silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. *Mol Cell Proteomics* 2012; 11:M111.014647.
29. Conti JA, Kendall TJ, Bateman A, et al. The desmoplastic reaction surrounding hepatic colorectal adenocarcinoma metastases aids tumor growth and survival via alphav integrin ligation. *Clin Cancer Res* 2008; 14:6405–6413.
30. Schober M, Jesenofsky R, Faissner R, et al. Desmoplasia and chemoresistance in pancreatic cancer. *Cancers* 2014; 6:2137–2154.
31. Whatcott CJ, Diep CH, Jiang P, et al. Desmoplasia in primary tumors and metastatic lesions of pancreatic cancer. *Clin Cancer Res* 2015; 21:3561–3568.
32. Zode GS, Clark AF and Wordinger RJ. Bone morphogenetic protein 4 inhibits TGF-beta2 stimulation of extracellular matrix proteins in optic nerve head cells: role of gremlin in ECM modulation. *Glia* 2009; 57(7): 755–766.
33. Porsch H, Bernert B, Mehić M, et al. Efficient TGFβ-induced epithelial-mesenchymal transition depends on hyaluronan synthase has2. *Oncogene* 2013; 32:4355–4365.
34. García R, Merino D, Gómez JM, et al. Extracellular heat shock protein 90 binding to TGFβ receptor I participates in TGFβ-mediated collagen production in myocardial fibroblasts. *Cell Signal* 2016; 28:1563–1579.

35. Yan M and Jurasz P. The role of platelets in the tumor microenvironment: from solid tumors to leukemia. *Biochim Biophys Acta Mol Cell Res* 2016; 1863:392–400.
36. Gkolfinopoulos S, Jones RL and Constantinidou A. The emerging role of platelets in the formation of the micrometastatic niche: current evidence and future perspectives. *Front Oncol* 2020; 10:374.
37. Provenzano PP, Inman DR, Eliceiri KW, et al. Collagen density promotes mammary tumor initiation and progression. *BMC Med* 2008; 6(1): 11.
38. Mammoto T, Jiang A, Jiang E, et al. Role of collagen matrix in tumor angiogenesis and glioblastoma multiforme progression. *Am J Pathol* 2013; 183:1293–1305.
39. Provenzano PP and Hingorani SR. Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer. *Br J Cancer* 2013; 108(1): 1–8.
40. Deak SB, Glaug MR, Pierce RA, et al. Desmoplasia in benign and malignant breast disease is characterized by alterations in level of mRNAs coding for types I and III procollagen. *Matrix* 1991; 11:252–258.
41. Kauppila S, Stenbäck F, Risteli J, et al. Aberrant type I and type III collagen gene expression in human breast cancer in vivo. *J Pathol* 1998; 186:262–268.
42. Barsky SH, Rao CN, Grotendorst GR, et al. Increased content of type V collagen in desmoplasia of human breast carcinoma. *Am J Pathol* 1982; 108:276–283.
43. Ajeti V, Nadiarnykh O, Ponik SM, et al. Structural changes in mixed col I/Col V collagen gels probed by SHG microscopy: implications for probing stromal alterations in human breast cancer. *Biomed Opt Express* 2011; 2:2307–2316.
44. Pucci-Minafra I and Luparello C. Type V/type I collagen interactions in vitro and growth-inhibitory effect of hybrid substrates on 8701-BC carcinoma cells. *J Submicrosc Cytol Pathol* 1991; 23:67–74.
45. Bar JK, Grelewski P, Popiela A, et al. Type IV collagen and CD44v6 expression in benign, malignant primary and metastatic ovarian tumors: correlation with Ki-67 and p53 immunoreactivity. *Gynecol Oncol* 2004; 95:23–31.
46. van Kempen LC, Rijntjes J, Mamor-Cornelissen I, et al. Type I collagen expression contributes to angiogenesis and the development of deeply invasive cutaneous melanoma. *Int J Cancer* 2008; 122:1019–1029.
47. Miskolczi Z, Smith MP, Rowling EJ, et al. Collagen abundance controls melanoma phenotypes through lineage-specific microenvironment sensing. *Oncogene* 2018; 37:3166–3182.
48. Reid SE, Kay EJ, Neilson LJ, et al. Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. *EMBO J* 2017; 36:2373–2389.
49. Grasset EM, Bertero T, Bozec A, et al. Matrix stiffening and EGFR cooperate to promote the collective invasion of cancer cells. *Cancer Res* 2018; 78:5229–5242.
50. Levental KR, Yu H, Kass L, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 2009; 139:891–906.
51. Gkretsi V and Stylianopoulos T. Cell adhesion and matrix stiffness: coordinating cancer cell invasion and metastasis. *Front Oncol* 2018; 8:145.
52. Katara GK, Kulshrestha A, Mao L, et al. Mammary epithelium-specific inactivation of V-ATPase reduces stiffness of extracellular matrix and enhances metastasis of breast cancer. *Mol Oncol* 2018; 12:208–223.
53. Tian H, Lyu Y, Yang YG, et al. Humanized rodent models for cancer research. *Front Oncol* 2020; 10:1696.
54. Hoffmann N, Fernández V, Pereira RC, et al. A Xenotransplant model of human brain tumors in wild-type mice. *iScience* 2020; 23(1): 100813.
55. Wishart AL, Conner SJ, Guarin JR, et al. Decellularized extracellular matrix scaffolds identify full-length collagen VI as a driver of breast cancer cell invasion in obesity and metastasis. *Sci Adv* 2020; 6:eabc3175. https://www.science.org.
56. Lü W-D, Zhang L, Wu CL, et al. Development of an acellular tumor extracellular matrix as a three-dimensional scaffold for tumor engineering. *PLoS One* 2014; 9:e103672.
57. Lv Y, Wang H, Li G, et al. Three-dimensional decellularized tumor extracellular matrices with different stiffness as bioengineered tumor scaffolds. *Bioact Mater* 2021; 6:2767–2782.
58. Matossian MD, Burks HE, Bowles AC, et al. A novel patient-derived xenograft model for claudin-low triple-negative breast cancer. *Breast Cancer Res Treat* 2018; 169:381–390.
59. Landberg G, Fitzpatrick P, Isakson P, et al. Patient-derived scaffolds uncover breast cancer promoting properties of the microenvironment. *Biomaterials* 2020; 235:119705.
60. Gustafsson A, Garre E, Leiva MC, et al. Patient-derived scaffolds as a drug-testing platform for endocrine therapies in breast cancer. *Sci Rep* 2021; 11(1): 13334.
61. Parkinson GT, Salerno S, Ranji P, et al. Patient-derived scaffolds as a model of colorectal cancer. *Cancer Med* 2021; 10:867–882.
62. Christenson JL, Butterfield KT, Spoelstra NS, et al. MMTV-PyMT and derived met-1 mouse mammary tumor cells as models for studying the role of the androgen receptor in triple-negative breast cancer progression. *Horm Cancer* 2017; 8:69–77.
63. Saldin LT, Klimak M, Hill RC, et al. The effect of normal, metaplastic, and neoplastic esophageal extracellular matrix upon macrophage activation. *J Immunol Regen Med* 2021; 13:100037.
64. Hoshiba T and Tanaka M. Decellularized matrices as in vitro models of extracellular matrix in tumor tissues at different malignant levels: mechanism of 5-fluorouracil resistance in colorectal tumor cells. *Biochim Biophys Acta Mol Cell Res* 2016; 1863(11): 2749–2757.
65. Burns JS, Kristiansen M, Kristensen LP, et al. Decellularized matrix from tumorigenic human mesenchymal stem cells promotes neovascularization with galectin-1 dependent endothelial interaction. *PLoS One* 2011; 6:e21888.
66. Vuoristo S, Toivonen S, Weltner J, et al. A novel feeder-free culture system for human pluripotent stem cell culture and induced pluripotent stem cell derivation. *PLoS One* 2013; 8:e76205.
67. Hielscher A, Qiu C, Porterfield J, et al. Hypoxia affects the structure of breast cancer cell-derived matrix to support angiogenic responses of endothelial cells. *J Carcinog Mutagen* 2013; Suppl 13:005.
68. Hoshiba T and Tanaka M. Breast cancer cell behaviors on staged tumorigenesis-mimicking matrices derived from tumor cells at various malignant stages. *Biochem Biophys Res Commun* 2013; 439:291–296.
69. Wang Y, Zhang T, Guo L, et al. Stromal extracellular matrix is a microenvironmental cue promoting resistance to EGFR

tyrosine kinase inhibitors in lung cancer cells. *Int J Biochem Cell Biol* 2019; 106:96–106.
70. Nayak B, Balachander GM, Manjunath S, et al. Tissue mimetic 3D scaffold for breast tumor-derived organoid culture toward personalized chemotherapy. *Colloids Surf B Biointerfaces* 2019; 180:334–343.
71. Cozzi E, Bosio E, Seveso M, et al. Xenotransplantation as a model of integrated, multidisciplinary research. *Organogenesis* 2009; 5:288–296.
72. Spade DJ, McDonnell EV, Heger NE, et al. Xenotransplantation models to study the effects of toxicants on human fetal tissues. *Birth Defects Res B Dev Reprod Toxicol* 2014; 101:410–422.
73. Martinez A, Buckley MS, Scalise CB, et al. Utilization of a 3-D tissue engineered model to investigate the effects of perfusion on gynecologic cancer biology. *J Tissue Eng* 2021; 12:20417314211055015.
74. Stamati K, Redondo PA, Nyga A, et al. The anti-angiogenic tyrosine kinase inhibitor pazopanib kills cancer cells and disrupts endothelial networks in biomimetic three-dimensional renal tumouroids. *J Tissue Eng* 2020; 11:2041731420920597.
75. Gilpin A and Yang Y. Decellularization Strategies for regenerative medicine: from processing techniques to applications. *Biomed Res Int* 2017; 2017(2017): 9831534.
76. Mendibil U, Ruiz-Hernandez R, Retegi-Carrion S, et al. Tissue-specific decellularization methods: rationale and strategies to achieve regenerative compounds. *Int J Mol Sci* 2020; 21:1–29.
77. D'Angelo E, Natarajan D, Sensi F, et al. Patient-derived scaffolds of colorectal cancer metastases as an organotypic 3D model of the liver metastatic microenvironment. *Cancers* 2020; 12.
78. Pinto ML, Rios E, Silva AC, et al. Decellularized human colorectal cancer matrices polarize macrophages towards an anti-inflammatory phenotype promoting cancer cell invasion via CCL18. *Biomaterials* 2017; 124:211–224.
79. Funamoto S, Nam K, Kimura T, et al. The use of high-hydrostatic pressure treatment to decellularize blood vessels. *Biomaterials* 2010; 31:3590–3595.
80. Halfwerk FR, Rouwkema J, Gossen JA, et al. Supercritical carbon dioxide decellularised pericardium: mechanical and structural characterisation for applications in cardio-thoracic surgery. *J Mech Behav Biomed Mater* 2018; 77:400–407.
81. Seo Y, Jung Y and Kim SH. Decellularized heart ECM hydrogel using supercritical carbon dioxide for improved angiogenesis. *Acta Biomater* 2018; 67:270–281.
82. Wang JK, Luo B, Guneta V, et al. Supercritical carbon dioxide extracted extracellular matrix material from adipose tissue. *Mater Sci Eng C* 2017; 75:349–358.
83. Aguado BA, Caffe JR, Nanavati D, et al. Extracellular matrix mediators of metastatic cell colonization characterized using scaffold mimics of the pre-metastatic niche. *Acta Biomater* 2016; 33:13–24.
84. Genovese L, Zawada L, Tosoni A, et al. Cellular localization, invasion, and turnover are differently influenced by healthy and tumor-derived extracellular matrix. *Tissue Eng Part A* 2014; 20:2005–2018.
85. Liu G, Wang B, Li S, et al. Human breast cancer decellularized scaffolds promote epithelial-to-mesenchymal transitions and stemness of breast cancer cells in vitro. *J Cell Physiol* 2019; 234:9447–9456.
86. Lin H-J, Wang W, Huang YY, et al. Decellularized lymph node scaffolding as a carrier for dendritic cells to induce anti-tumor immunity. *Pharmaceutics* 2019; 11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6920996/
87. Romero-López M, Trinh AL, Sobrino A, et al. Recapitulating the human tumor microenvironment: colon tumor-derived extracellular matrix promotes angiogenesis and tumor cell growth. *Biomaterials* 2017; 116:118–129.
88. Hashimoto Y, Funamoto S, Sasaki S, et al. Preparation and characterization of decellularized cornea using high-hydrostatic pressurization for corneal tissue engineering. *Biomaterials* 2010; 31:3941–3948.
89. Piccoli M, D'Angelo E, Crotti S, et al. Decellularized colorectal cancer matrix as bioactive microenvironment for in vitro 3D cancer research. *J Cell Physiol* 2018; 233:5937–5948.
90. Nouri Barkestani M, Naserian S, Uzan G, et al. Post-decellularization techniques ameliorate cartilage decellularization process for tissue engineering applications. *J Tissue Eng* 2021; 12:2041731420983562.
91. Hochman-Mendez C, Pereira de Campos DB, Pinto RS, et al. Tissue-engineered human embryonic stem cell-containing cardiac patches: evaluating recellularization of decellularized matrix. *J Tissue Eng* 2020; 11:2041731420921482.
92. Levin A, Sharma V, Hook L, et al. The importance of factorial design in tissue engineering and biomaterials science: Optimisation of cell seeding efficiency on dermal scaffolds as a case study. *J Tissue Eng* 2018; 9:2041731418781696.
93. Carpino G, Overi D, Melandro F, et al. Matrisome analysis of intrahepatic cholangiocarcinoma unveils a peculiar cancer-associated extracellular matrix structure. *Clin Proteomics* 2019; 16:37.
94. Coletta S, Lonardi S, Sensi F, et al. Tumor cells and the extracellular matrix dictate the pro-tumoral profile of macrophages in CRC. *Cancers* 2021; 13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8533926/
95. Mazza G, Telese A, Al-Akkad W, et al. Cirrhotic human liver extracellular matrix 3D scaffolds promote smad-dependent TGF-β1 epithelial mesenchymal transition. *Cells* 2019; 9:83.
96. Helal-Neto E, Brandão-Costa RM, Saldanha-Gama R, et al. Priming endothelial cells with a melanoma-derived extracellular matrix triggers the activation of αvβ3/VEGFR2 axis. *J Cell Physiol* 2016; 231:2464–2473.
97. Dodson RM, Weiss MJ, Cosgrove D, et al. Intrahepatic cholangiocarcinoma: management options and emerging therapies. *J Am Coll Surg* 2013; 217:736–750.e4.
98. Praktiknjo M, Lehmann J, Nielsen MJ, et al. Acute decompensation boosts hepatic collagen type III deposition and deteriorates experimental and human cirrhosis. *Hepatol Commun* 2018; 2:211–222.
99. Liu X, Fang J, Huang S, et al. Tumor-on-a-chip: from bioinspired design to biomedical application. *Microsyst Nanoeng* 2021; 7:50.
100. Plou J, Juste-Lanas Y, Olivares V, et al. From individual to collective 3D cancer dissemination: roles of collagen concentration and TGF-β. *Sci Rep* 2018; 8:12723.
101. Sontheimer-Phelps A, Hassell BA and Ingber DE. Modelling cancer in microfluidic human organs-on-chips. *Nat Rev Cancer* 2019; 19:65–81.
102. Zhang C, Yang Z, Dong DL, et al. 3D culture technologies of cancer stem cells: promising ex vivo tumor models. *J Tissue Eng* 2020; 11:2041731420933407.