Extracellular matrix-based materials for regenerative medicine

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Abstract | In tissue engineering and regenerative medicine, a biomaterial provides mechanical support and biochemical signals to encourage cell attachment and modulate cell behaviour. Nature's template for a biomaterial is the extracellular matrix (ECM). The ECM contains intrinsic biochemical and mechanical cues that regulate cell phenotype and function in development, in homeostasis and in response to injury. The use of ECM-based materials in biomedical research has advanced from coating cell culture plates with purified ECM components to the design of ECM-mimicking biomaterials and the engineering of decellularized tissues aimed at recapitulating the dynamics, composition and structure of the ECM. In this Review, we highlight important matrix properties and functions in the context of tissue engineering and regenerative medicine, consider techniques such as proteomics for the investigation of matrix structure and composition and discuss different engineering strategies for the design of matrix-mimicking biomaterials. Tissue, whole organ and cell culture decellularization approaches are examined for their potential to preserve the tissue-specific biochemical composition and ultrastructure of the ECM and for the development of biomaterials that promote the formation of functional tissues in clinical applications. Finally, we investigate challenges and opportunities of ECM biomaterials for the design of organotypic models to study disease progression, for the ex vivo creation of engineered tissue and for the clinical translation of functional tissue reconstruction strategies in vivo.

The ability to generate functional tissues and organs as replacements for their damaged or diseased counterparts is a rapidly advancing pursuit in the field of tissue engineering and regenerative medicine. Although cell biologists have been isolating and culturing cells from living tissue since the beginning of the 20th century, these monolayer cell cultures were typically 2D (FIG. 1). Multicellular organisms require a 3D framework not only to provide structural integrity to the organism but also to denote functional tissue boundaries and delineate specific microenvironments[1]. Therefore, to engineer whole organs and tissues, it is necessary to integrate principles of cell biology with materials science and to grow cells and tissues in a 3D environement[2]. In fact, the tremendous progress in tissue engineering and regenerative medicine over the past few decades can be largely attributed to the development of biomaterials designed to exert mechanical and biochemical cues that guide cell behaviour, with the general strategy to combine cells with 3D biodegradable scaffolds to create replacement tissues.

Multicellular organisms have been operating within this design paradigm since early metazoan evolution. The evolutionary transition from unicellular to multicellular organisms has enabled cells to cohesively cooperate as a group to perform complex tasks. A key step in the evolutionary process to multicellularity was the emergence of genes coding for structural and functional molecules that can be secreted by cells and arranged into a 3D extracellular matrix (ECM)[3]. The ECM not only provides a physical scaffold for maintaining the structural integrity of multicellular organisms but also serves as a reservoir for biochemical and biophysical signals to support cell survival, organization and differentiation[1] (FIG. 2a). Many ECM proteins originated during early metazoan evolution and have remained highly conserved in both vertebrate and invertebrate species, underscoring the importance of these ECM molecules in multicellular life[4,5]. The necessity of an ECM for metazoan biology is still evident in present-day embryonic development. Stem cells deposit ECM molecules at the very earliest stages of embryogenesis[6]. For example, the structural ECM molecule laminin is expressed at the 16-cell stage in the developing mouse embryo, closely followed by collagen type IV expression in the early blastocyst[7].

The differentiation and migration events guiding cells to divide and integrate into tissues and organs with distinct functions are accompanied by the continuous and dynamic remodelling of the ECM into tissue-specific 3D

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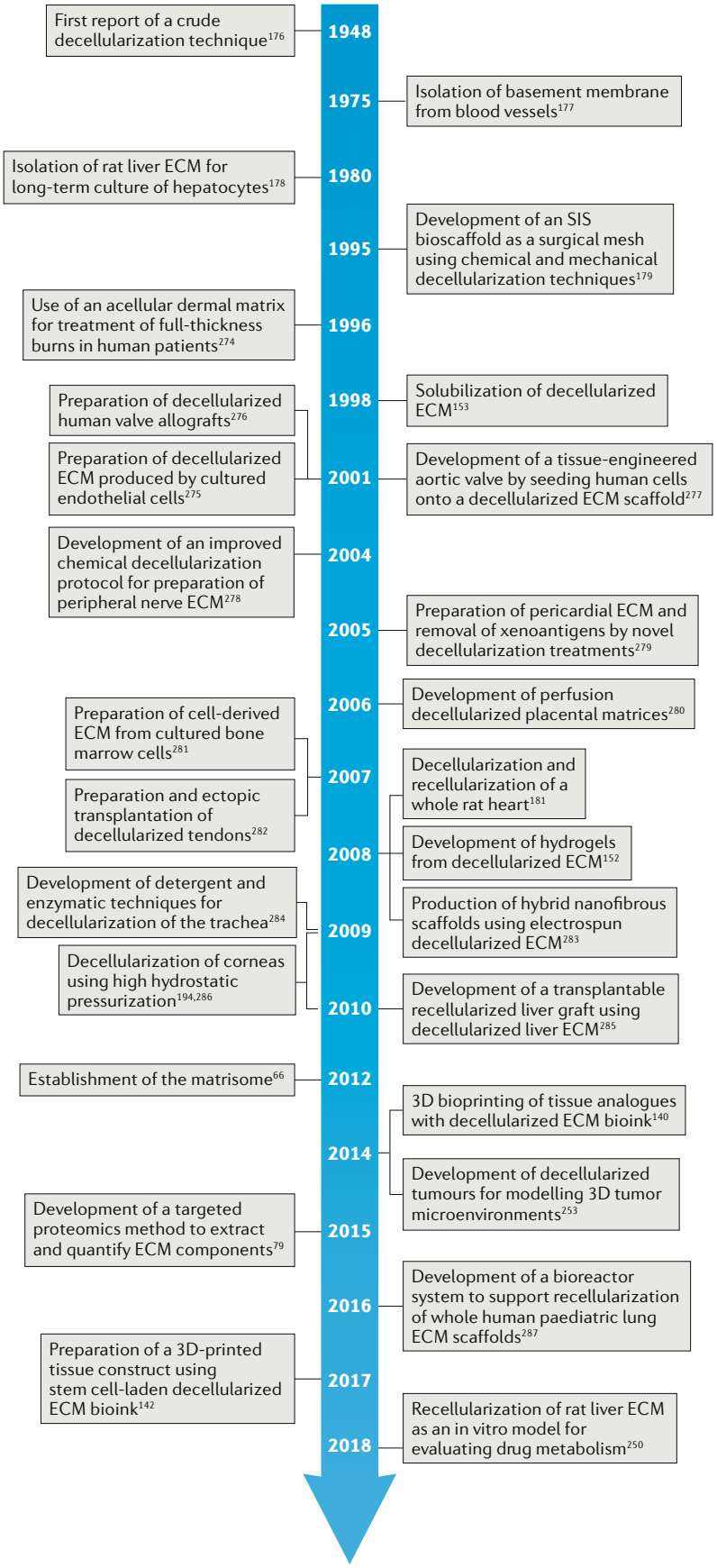

Fig. 1 | **Milestones of decellularization technologies.** ECM, extracellular matrix; SIS, small intestine submucosa[274–287].

architectures and compositions[8]. Thus, the resident cells of each tissue are responsible for and responsive to the ECM in a process referred to as dynamic reciprocity or bidirectional crosstalk between the cell and its environment[9–11]. Cells modify their secreted ECM products in response to various stimuli, including mechanical cues, oxygen and nutrient concentration, and many other factors that constitute the microenvironmental niche[12]. In turn, the ECM sends mechanical and biochemical signals to resident cells through the engagement of cell surface receptors, subsequent activation of intracellular signalling cascades and, ultimately, changes in gene expression and cell phenotype[1,9]. The synthesis and secretion of ECM molecules by resident cell types continue throughout life in both healthy and diseased physiological states[13] and regulate numerous biological processes, including stem cell differentiation[14,15], angiogenesis[13,16], innervation[17] and wound healing[18–20]. Owing to its evolutionarily conserved composition and impact on both embryonic development and cellular and organ homeostasis, the ECM constitutes an ideal biomaterial not only as an inductive substrate to promote the repair of damaged tissue within the body but also as a scaffold for the engineering of whole tissues and organs on the benchtop.

Numerous ECM analogues have been developed, mimicking a physiological 3D microenvironment, to support cellular function, including synthetic scaffolds derived from polymeric substrates (for example, polycaprolactone, polyethylene glycol and polyglycolic acid)[21], hydrogels synthesized from crosslinked hydrophilic polymers (for example, polyacrylic acid, polyethylene glycol and polyvinyl alcohol)[22,23], ceramic-based scaffolds fabricated from hydroxyapatite or tricalcium phosphate[24] and natural biopolymers derived from a diverse array of species, including alginate (derived from algae)[25], chitosan (derived from arthropod exoskeletons)[26], cellulose (derived from plants)[27] and silk fibroin (derived from the *Bombyx mori* silkworm)[28]. Although these ECM analogues have been extensively evaluated for tissue engineering applications[29], they lack the complex biochemical properties and 3D ultrastructure of native mammalian ECM. The past decade has witnessed substantial progress towards the next generation of ECM-based biomaterials, including the fractionation and characterization of ECM components and the development of decellularization techniques for the preservation of native mammalian ECM structure and composition.

In this Review, we examine biomaterials derived from mammalian ECM and its components and highlight major applications in tissue engineering and regenerative medicine. We discuss ECM biomaterials for their potential to regenerate and repair tissues and investigate how ECM biomaterials have progressed from benchtop and preclinical studies to clinically translatable therapies with over 20 years of regulatory approvals, clinical use and reimbursement history.

Matrix function and composition

The ECM is a fibrous network of proteins, proteoglycans and glycosaminoglycans arranged in a tissue-specific 3D architecture that provides cells and tissues with topographical signalling cues, structural and elastic

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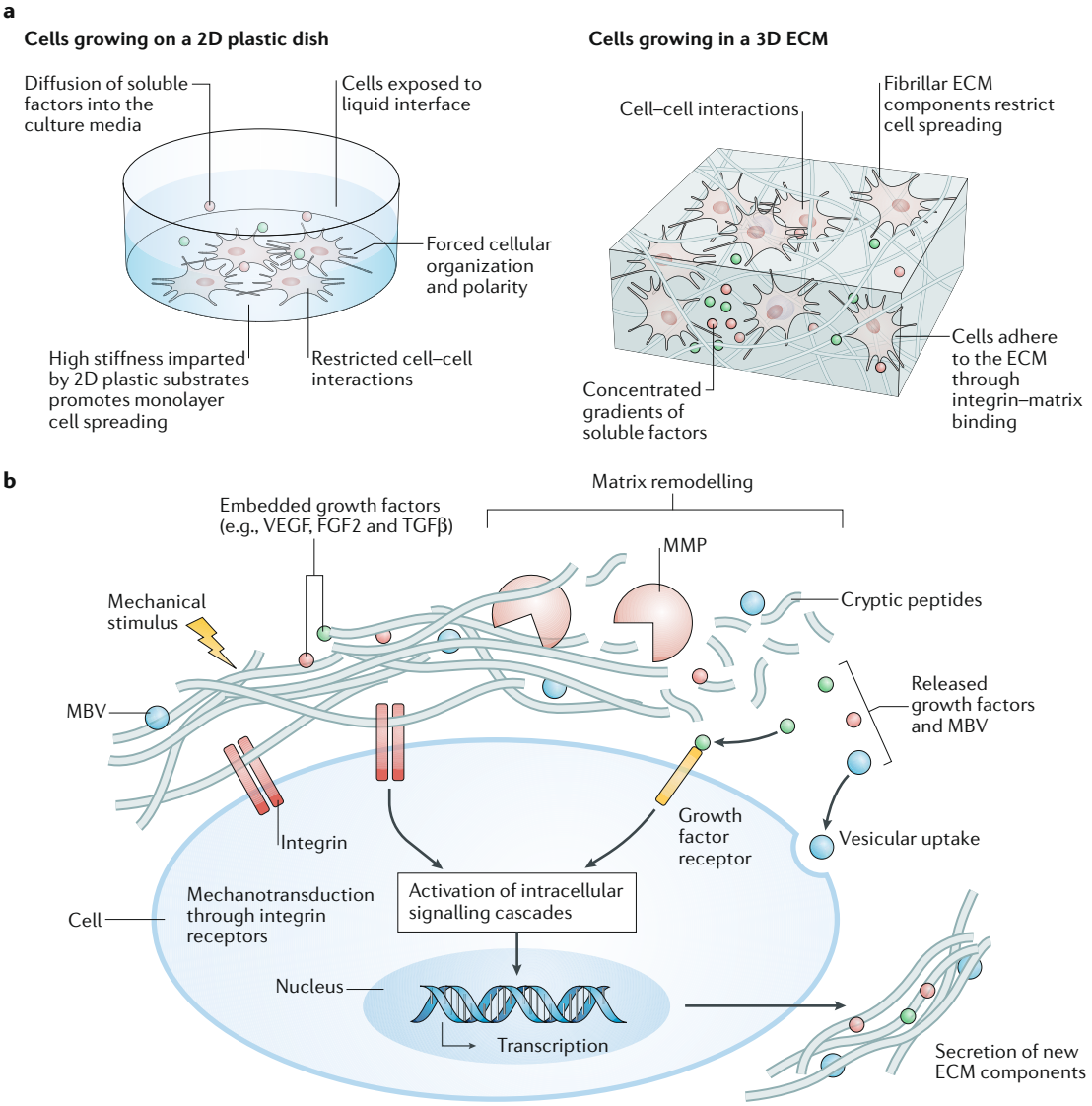

Fig. 2 | **Cell–extracellular matrix interactions and matrix remodelling. a** | 2D plastic substrates (left) restrict cell attachment in a planar direction and force cells into an apical–basal polarity but allow diffusion of secreted soluble factors in the culture medium. By contrast, 3D extracellular matrix (ECM) substrates (right) enable cell attachment in both planar and perpendicular directions without restricting cell polarity. The discrete matrix fibrils sterically hinder the spreading of cells and contribute to the sequestration of secreted growth factors into concentrated gradients within the matrix. **b** | Cells interact with their environment through integrin and growth factor receptors. Cells convert mechanical stimuli from the ECM into biochemical activity through the binding and activation of integrin receptors, resulting in the activation of intracellular signalling pathways, activation of gene transcription and synthesis and secretion of ECM components. During matrix remodelling, proteolytic degradation induced by matrix metalloproteinases (MMPs) results in the release of tethered growth factors and matrix-bound nanovesicles (MBVs), as well as the production of cryptic peptides. The released bioactive components can interact with cells to promote diverse cellular functions such as proliferation, migration and differentiation. FGF2, fibroblast growth factor 2; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

properties and a medium for diffusion and convection of nutrients and oxygen[30–33]. In addition to its structural and mechanical functions, the ECM also serves as an adhesive substrate not only for cell attachment and migration[1] but also for the sequestration of growth factors and morphogens[34,35], resulting in the establishment of specialized local microenvironments that contribute to the differentiation and maintenance of tissue-specific cell phenotypes and functions[36,37]. The mechanical and biochemical cues provided by the ECM are deciphered by various cell surface receptors[38], among which the most widely studied ECM receptors are integrins[39,40]. Integrin–ECM interactions trigger intracellular signalling cascades resulting in the expression of genes that regulate cell survival, proliferation, differentiation and apoptosis[41]. Reciprocally, resident cells mediate the constant and complex rebuilding and remodelling of the ECM through synthesis, biochemical modification (for example, crosslinking), degradation and release of bioactive molecules, as well as reassembly of ECM

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components[8] (FIG. 2b). These processes are tightly regulated during embryonic and tissue development[8], homeostasis[42,43] and ageing[44,45], as well as in response to injury[20,46]. Importantly, dysregulation of these processes has been shown to initiate and promote pathological conditions and disease progression[8,47]. For example, excessive ECM deposition can lead to pathological fibrosis[48], and abnormal ECM degradation has been associated with cardiac dysfunction[49], osteoarthritis[50] and chronic obstructive pulmonary disease[51].

The ECM consists of biochemical molecules secreted by the resident cells of the tissue or organ, and thus, the composition varies depending on the physiological requirements of a particular tissue[52]. The most abundant and well-studied protein of mammalian ECM is collagen, which accounts for nearly 90% of the dry weight of most tissues and organs and is responsible for maintaining the structural integrity of the tissue[53]. Although type I collagen is the major structural protein present within tissues, 28 distinct types of collagen have been identified thus far[54,55]. Other abundant structural molecules include glycosaminoglycans (for example, chondroitin sulfate, heparin and hyaluronic acid (HA)), which promote water retention and compressive resistance[56], and adhesion molecules, such as laminin[57], fibronectin[58] and tenascin-c[59], which function as a molecular glue to reinforce the structural network. Many of these molecules are bifunctional; for example, structural ECM molecules have been shown to also promote cell attachment through distinct peptide domains[60], such as the Arg-Gly-Asp (RGD) and Arg-Glu-Asp-Val (REDV) sequences in fibronectin[61,62] and the Val-Ala-Pro-Gly (VAPG) domain in elastin[63].

Various ECM-modifying proteins, such as transglutaminases, lysyl oxidases and hydroxylases, enable crosslinking and thus strengthen ECM biopolymers[64,65], whereas proteolytic enzymes[8,66] (for example, matrix metalloproteinases (MMPs), elastases, cathepsins and serine proteases) play key roles in ECM turnover, in the release of ECM-bound growth factors and in the exposure of matricryptic peptides[17,67–69], which mediate diverse cellular functions, such as chemotaxis[70,71], mitogenesis[72], angiogenesis[73] and inflammation[74]. Furthermore, matrix-bound nanovesicles (MBVs) are embedded within the fibrillar ECM network[75] (FIG. 2b). MBVs are nanometre-sized, membranous vesicles that contain biologically active signalling molecules (for example, proteins, microRNA and lipids) that mediate stem cell differentiation and macrophage activation[75–77].

Advanced proteomic techniques and bioinformatics tools can be applied to characterize ECM compositions and to identify ECM protein signatures, termed the matrisome[66]. This targeted proteomics approach has been facilitated by the development of protocols for extracting insoluble fibrillar components, such as collagen and elastin, from the ECM and by the generation of stable isotope-labelled ECM peptides for absolute protein quantification[78–80]. The combined use of bioinformatics and mass spectrometry has enabled the identification of ECM protein signatures of many healthy and diseased tissues and has uncovered tissue-specific ECM proteins and novel biomarkers for disease progression[81–83]. Although our understanding of ECM structure and composition is an ongoing pursuit, the contributions of bioinformatics and targeted proteomics have greatly expanded the frontiers of ECM biomaterial design and fabrication.

Matrix biomaterials

Biomaterials for functional tissue repair can be broadly categorized into two subtypes: naturally occurring and synthetic materials. Naturally occurring materials are generally processed from whole ECMs or from purified, individual ECM components (for example, collagen, laminin, fibronectin and silk), whereas synthetic materials are typically composed of manufactured polymers, chemicals, metals or other synthetically derived substrates. Both material types have distinct advantages and disadvantages. For example, synthetic materials can be precisely and consistently manufactured and therefore show minimal variability. Moreover, their properties, such as mechanical strength and degradation profile, can be readily tuned, and multiple polymers can be integrated within one material[84]. However, synthetic materials, especially non-degradable materials, are associated with a pro-inflammatory host response[85–87]. By contrast, ECM-based biomaterials show greater variability owing to their biological source but have a more favourable pro-remodelling host immune response because they provide a natural, instructive microenvironmental niche for functional tissue remodelling[88,89]. Ideally, the engineering of ECM biomaterials combines the precision and control of synthetic material manufacturing and the beneficial bioactive properties of an ECM-based material to promote tissue remodelling in situ. Biomimetic (or biosynthetic) materials can be engineered using a variety of fabrication techniques, such as soft lithography, electrospinning and 3D printing, in the attempt to mimic the inherent cues of the ECM to combine the best of both material classes (FIG. 3).

Engineering of biomimetic materials

Incorporation of matrix molecules. Synthesized bioactive domains of ECM proteins, such as the RGD domain of fibronectin or the Val-Pro-Val-Gly-Val (VPVGV) domain of elastin, can promote the attachment of cells and growth factors to synthetic materials. These peptides can be physically or chemically crosslinked into a synthetic material to form a network or lattice-like structure that mimics native ECM[90,91]. For example, polyethylene glycol (PEG) chains containing plasmin and MMP substrates on one end and cell-adhesion peptides on the other end can be photopolymerized into hydrogels through stepwise crosslinking to control integrin-driven migration of fibroblasts or endothelial cells[92,93].

A better understanding of soluble ECM signalling molecules and adhesive substrates that regulate stem cell differentiation and cell fate during tissue development, homeostasis and wound healing has further enabled the synthetic design of ECM-mimicking instructive signals and factors, for example, the release of nerve growth factor beta (NGFβ) to control fetal brain cell survival and differentiation in vivo[94,95], the control of embryonic stem cell fate through topographic mimics of the ECM[96],

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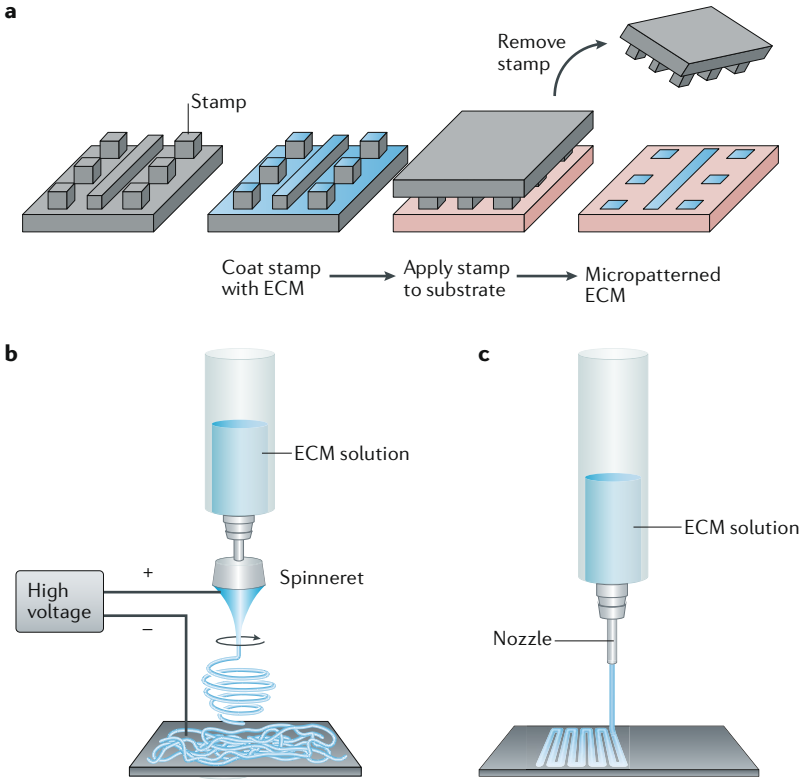

Fig. 3 | **Fabrication of extracellular matrix biomaterials. a** | Soft lithography. An elastomeric stamp is first coated with an extracellular matrix (ECM) material, which is then stamped onto a substrate surface to create micropatterns of the ECM material. **b** | Electrospinning. Nanofibrous networks are created from electrically charged jets of biopolymer solutions or ECM hydrogels that are deposited onto a grounded collector. **c** | 3D bioprinting. In extrusion-based 3D printing, hydrogels and colloids (bioinks) are deposited layer by layer to produce a 3D structure.

the use of bone morphogenic protein (BMP)-loaded materials to fill bone defects[97] and vascular endothelial growth factor (VEGF)-releasing materials to promote blood vessel growth at the material implant site[98]. Purified ECM components enriched at the intestinal crypt base[99] can also be incorporated in synthetic PEG hydrogels to improve intestinal stem cell (ISC) survival and proliferation[100], and maleimide-modified PEG macromers (PEG-4MAL) can be functionalized with RGD peptides to support growth and expansion of human intestinal organoids[101].

In addition to tailoring the biochemical and ultrastructural components of synthetic ECM materials, the dynamics of the ECM can be resembled by controlling the spatial arrangement and temporal release of signalling molecules, for example, through spatial patterning of self-assembling or boundary-forming signals, such as cadherins or ephrins[102], or through the multimodal and temporal release of growth factors. For example, the release of platelet-derived growth factor BB (PDGF-BB) and transforming growth factor beta 1 (TGFβ1) from PEGylated fibrin gels can be used to mimic vasculogenesis for capillary tube stabilization and mural cell differentiation[103]. Although synthetic matrices cannot capture the full complexity and dynamics of ECMs, the bottom-up approach of synthesizing biomimetic matrices is a powerful method to control cell behaviour and ultimately tissue growth, with the potential to provide key insights into the physiological processes that regulate ECM-mediated cell behaviour.

Micropatterning and self-assembly of matrix components. ECM composition and ultrastructure play important roles in guiding cell behaviour[104], and each organ and tissue has a distinct ECM ultrastructure, which can be recreated through micropatterning of ECM components onto synthetic materials. Photolithography, or light-based patterning, can be used to impart differential ECM protein deposition on the surface of a material with resolutions of 500–5000 µm (REF.[105]) but has several limitations, for example, the high cost of photolithographic equipment and of maintaining clean rooms[106]. By contrast, elastomeric stamping techniques have improved control mechanisms, can incorporate microchannels and microfluidics and are procedurally simple and inexpensive[106,107] (FIG. 3a). Elastomeric stamping enables the patterning of biochemical mosaics and gradients of ECM components. Furthermore, using nanofibre lithography, fibrous nanopatterned scaffolds can be fabricated with resolutions of 250–1000 nm (REF.[108]) to control cell adhesion through the regulation of integrin expression[108].

The composition, topography and mechanical properties of micropatterned ECM substrates affect cellular signalling pathways, and cells behave differently on micropatterned substrates than on 2D substrates. For example, the shape and phenotype of macrophages can be modulated by the elasticity and rigidity of substrates micropatterned with fibronectin, without exposure to exogenous cytokines[109]. Changes in macrophage gene expression and cytokine secretion profiles are mediated by mechanotransduction signalling pathways through actin polymerization and activation of stretch-sensitive ion channels[110], triggered by changes in substrate elasticity. Similarly, angiogenesis can be modulated by micropatterned substrates; the formation of blood vessels preferentially occurs in the convex parts of micropatterned vessel walls, where mechanical forces are highest[111,112]. Mechanical forces modulate several important cellular functions[113–116], including apoptosis[117], differentiation[118], RNA processing[119] and gene expression[120], emphasizing the role of the ECM structure, topography and mechanics for tissue remodelling. Improved imaging and data analysis techniques have revealed the remarkable complexity of the ECM architecture; however, a detailed mapping of the native ECM topography required for the exact recreation of the ECM structure by micropatterning remains elusive.

Alternatively, molecular self-assembly can be used as a bottom-up approach for the fabrication of substrates, inspired by nature's method of creating complex architectures through native protein self-assembly. Using this technique, nanopatterns can be engineered at a resolution of ~10 nm (REFS[90,121]), for example, to include nanowires, whose assembly can be electronically controlled[122,123], by magnetic assembly of engineered bacteriophages[124] or to generate complex biological structures, such as sheets and ribbons[125]. The continuous development of self-assembled structures will help drive the next generation of micropatterned surfaces and may provide important insights into the interaction of the ECM with

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cell surface proteins, intracellular protein trafficking and the generation of genetic materials[90].

Electrospinning. Electrospinning enables the fabrication of ECM scaffolds and synthetic materials as woven, multifilament braided and knitted meshes. Electrospinning is a technique in which a polymer or ECM-based solution is forced through an electrostatic field to generate fibrous networks at diameters ranging from a few nanometres to ≥1 micrometre[126,127] (FIG. 3b). Electrospinning is a versatile and relatively inexpensive approach to create scaffolds that mimic the native ECM architecture. The pore sizes and network of interwoven fibres enable cellular infiltration and differentiation. A broad range of synthetic polymers, including polycaprolactone (PCL), polylactic-co-glycolic acid (PLGA) and polyurethanes, can be electrospun into scaffolds at large scales, allowing the diffusion of nutrients and growth factors. However, materials fabricated by electrospinning are limited by poor cell growth and differentiation, potential cytotoxicity due to residues after production[128] and inadequate mechanical strength[126]. Moreover, owing to often limited cell infiltration, electrospun scaffolds behave more like 2D substrates than 3D microenvironments. These issues can be addressed by selecting an appropriate solvent to minimize toxic residues, by optimizing the pore size, by reducing the packing density of the fibres and by including biological factors into the spinning process[129], such as cells or ECM components (FIG. 3b). For example, a fibrin-based electrospun nerve cable recapitulates the structure and function of a native fibrin nerve cable and thus supports Schwann cell adhesion, migration and motor neuron function[130]. Albumin-based electrospun cardiac patches can be generated with multiple layers to support the propagation of electrical signals[131]. Moreover, ECM hydrogels[132,133] can be electrospun to better resemble the composition and properties of native ECM and to improve the incorporation of cells in the process. Electrospinning technologies that include a combination of bioactive factors to support cell viability are promising for whole organ engineering, which requires viable cell seeding before implantation.

3D bioprinting. 3D bioprinting is a versatile method that uses a layer-by-layer approach to efficiently produce tissue constructs, with the potential to control ECM architecture, composition, pore size and surface modification[134]. Direct ink writing (DIW) is a basic extrusion-based 3D printing technique using hydrogels and colloids. DIW is sensitive enough to enable the printing of biofriendly inks and cells, expanding the range of printable materials and allowing for higher fidelity between the models generated with the help of computed tomography (CT) and magnetic resonance imaging (MRI), and the final product[135,136]. Therefore, 3D printing technologies can be applied for the production of patient-specific materials and custom lab tools to analyse cell behaviour. Synthetic materials were first used for the 3D printing of scaffolds for clinical applications, for example, polyetherketone bone plates for cranial defect repair[137] and polycaprolactone for tracheal repair[138]. However, the use of ECM-based materials has improved the biocompatibility of 3D-printed materials[139,140]. For example, 3D bioprinting by the additive manufacturing of alginate, HA, fibrin and collagen can be applied to generate complex 3D scaffolds for a wide range of physiological structures. These printed scaffolds do not collapse or deform; they are anisotropic, mimicking the microstructure of native tissues; and cell viability is retained throughout the printing process[141]. Similarly, bioinks derived from the ECM of fat, cartilage and heart tissue can be 3D printed to generate a cardiac patch that incorporates human cardiac progenitor cells and mesenchymal stem cells[140,142]. Liver-derived ECM can further be 3D printed to generate liver constructs that improve stem cell differentiation and hepatocellular functions[143]. However, technical improvements are required to better control the structural integrity and localization of bioactive molecules within 3D-printed ECM-based biomaterials[134].

Hydrogels. One of the major advancements in the engineering of ECM-based biomaterials is the possibility to form ECM hydrogels. Hydrogels are injectable and compatible with a variety of fabrication technologies, including 3D printing, micropatterning and electrospinning, and thus expand the clinical applicability of ECM-based biomaterials. Hydrogels are hydrated polymers or materials with ≥30% (v/w) water content that maintain their structural integrity through crosslinks between their constituents[144]. Hydrogels are commonly composed of synthetic polymers[145], such as poly(propylene fumarate-co-ethylene glycol) [P(PF-co-EG)], poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA) and polyethylene oxide (PEO). Single ECM components can also form hydrogels, for example, alginate, collagen, elastin and HA[146]. Furthermore, multicomponent hydrogel networks of polymer-functionalized ECM molecules can be designed to better mimic the physical properties of native tissues[147], for example, incorporation of methacrylated HA within a fibrin hydrogel[148], interpenetrating polymer networks of photocrosslinked HA and semi-interpenetrating collagen[149] and tri-component networks composed of collagen, methacrylate-modified chondroitin sulfate and HA[150].

Cell-derived ECM hydrogels, such as Matrigel, have been widely used to support in vitro cell culture in a 3D microenvironment[151]. Moreover, ECM derived from decellularized tissue can be used to generate hydrogels through enzymatic solubilization of the ECM and neutralization to physiological pH and temperature[152,153]. These ECM hydrogels are biocompatible and have been used in preclinical applications, for example, for the treatment of ulcerative colitis[154], traumatic brain injury[155] and stroke[156,157]. ECM hydrogels derived from decellularized tissues can also accurately recapitulate the in vivo microenvironment of a stem cell niche. Therefore, ECM hydrogels are an attractive substrate for 3D organoid culture to promote proliferation and differentiation of stem cells[158,159] and as coatings for polypropylene mesh materials to mitigate a negative host immune response[152,160]. ECM hydrogels can be generated from any tissue in the body though decellularization; however, the biochemical, topological and viscoelastic properties

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of ECM hydrogels[161] depend on the tissue type and decellularization protocol.

ECM hydrogels can be used as coatings for tissue culture plastic, as 3D gels for 3D cell culture and as media supplements to support the culture and maturation of cell lines, primary cells and stem or progenitor cells[161–163]. Decellularized ECM hydrogels support cell viability and differentiation as well as or even better than other commonly used substrates, such as Matrigel, or individual ECM components, such as collagen hydrogels[143,164–172]. ECM hydrogels delivered through a catheter are being investigated in clinical trials to treat ischaemic injury, myocardial infarction and peripheral artery disease[173,174]. Moreover, ECM hydrogels mitigate the expression of pro-inflammatory and pro-apoptotic genes and promote blood vessel formation and stem and progenitor cell recruitment and differentiation in the heart, and thus have been proposed to be able to directly promote endogenous repair of the myocardium[175]. To further expand the clinical use of ECM hydrogels, their concentration-dependent rheological properties have to be determined, appropriate source tissues have to be selected for specific clinical applications, and sterilization methods have to be optimized.

Decellularized matrix bioscaffolds

Given the complexity and incomplete understanding of ECM composition and structure, designing and fabricating an ECM scaffold that fully mimics the biochemistry and architecture of native tissue ECM are currently not possible. However, decellularization of whole tissues and organs by removing all cellular components provides an alternative method for harvesting an ECM with tissue-specific 3D morphology, microarchitecture and molecular composition. The first scientific report demonstrating a crude decellularization technique was published in 1948 (REF.[176]). In this early study, researchers showed that acellular homogenates can be prepared by pulverizing muscle tissue at −70 °C to remove cellular components. Following this first decellularization approach, isolation of the basement membrane of blood vessels[177] and isolation of the ECM of the liver[178] were reported. In the 1990s, mechanical and chemical decellularization techniques were developed to produce decellularized ECM scaffolds from tissues such as the skin and small intestine for biomedical applications[179,180] (FIG. 1). For example, an early preclinical study in a canine animal model showed that an ECM scaffold derived from decellularized small intestinal submucosa can be used as a biomaterial for the repair of a damaged Achilles tendon[179]. Similarly, acellular dermal matrices derived from decellularized skin showed promising results as an inductive substrate for tissue repair in the treatment of full-thickness burns[180]. These early pioneering studies demonstrated that a decellularized ECM scaffold can be used as a biomaterial to promote functional and constructive tissue remodelling following in vivo implantation. The decellularization techniques developed for processing planar tissues such as the dermis and small intestinal submucosa provided the basis for advanced decellularization techniques, for example, perfusion decellularization, which preserves not only the overall 3D ECM morphology but also an intact vascular tree, providing a route for the reseeding of site-specific cells. Perfusion decellularization was first demonstrated in 2008 with the decellularization and recellularization of a whole rat heart[181], followed by the lung, kidney and liver[182] (FIG. 1). The field is now also exploring targeted proteomic techniques to map the repertoire of ECM components present in decellularized ECM scaffolds to enable the design of ECM-mimicking biomaterials[81].

Decellularization methods. The goal of decellularization is to remove all cells and genetic material from a native ECM and to maintain the structural, biochemical and biomechanical properties of the ECM (FIG. 4). In general, decellularization techniques are tailored to preserve distinct physical and biochemical characteristics of a specific tissue, including thickness, ECM density and 3D configuration. Decellularization protocols have been described for almost every tissue in the body[183] and usually involve a combination of physical, chemical and enzymatic strategies[183–192]. Physical methods include freeze–thaw processing[193], hydrostatic pressure[194,195] and mechanical delamination of specific tissue layers[196]. Chemical reagents, such as peracetic acid[197,198], sodium hydroxide[199], hypotonic and hypertonic solutions[200,201], chelating reagents[184] and detergents such as sodium deoxycholate and sodium dodecyl sulfate (SDS)[184] are used to lyse cell membranes and to remove cytosolic and genomic material. Alcohols, such as methanol and acetone, are used for delipidization[202]. In addition, enzymes, such as proteases (for example, trypsin, dispase and thermolysin) and nucleases (RNase and DNase), digest cell debris and nucleic acids[184]. Importantly, both the selection of decellularization reagents and their application are dependent on the characteristics of the tissue of interest. For example, decellularized ECM scaffolds can be derived from a variety of tissues[184], for example, oesophagus, tendon, heart valves, skeletal muscle and trachea, by agitated immersion of the tissue in decellularization reagents. By contrast, luminal perfusion of decellularization reagents into hollow tissues can be applied to umbilical veins[203] and urinary bladder[204], and for retrograde perfusion into the vasculature of complex organs such as the heart[181], lung[205], liver[206] and kidney[207] to remove cells from the ECM. Alternatively, the use of supercritical carbon dioxide[208] and non-thermal irreversible electroporation[209,210] has been explored for tissue decellularization.

However, every decellularization method invariably disrupts the ECM to some degree. Furthermore, inefficient decellularization has detrimental effects on constructive tissue remodelling following implantation[199,211]. Therefore, there is a veritable balance between the preservation of the native ECM structure and composition and the removal of cellular and antigenic material, such as nucleic acids, membrane lipids and cytosolic proteins, because residual cellular components can elicit an adverse inflammatory response and inhibit constructive remodelling[212]. Moreover, processing methods used to increase the mechanical strength of decellularized ECM scaffolds, such as chemical crosslinking, can prevent degradation of the ECM material in vivo, resulting

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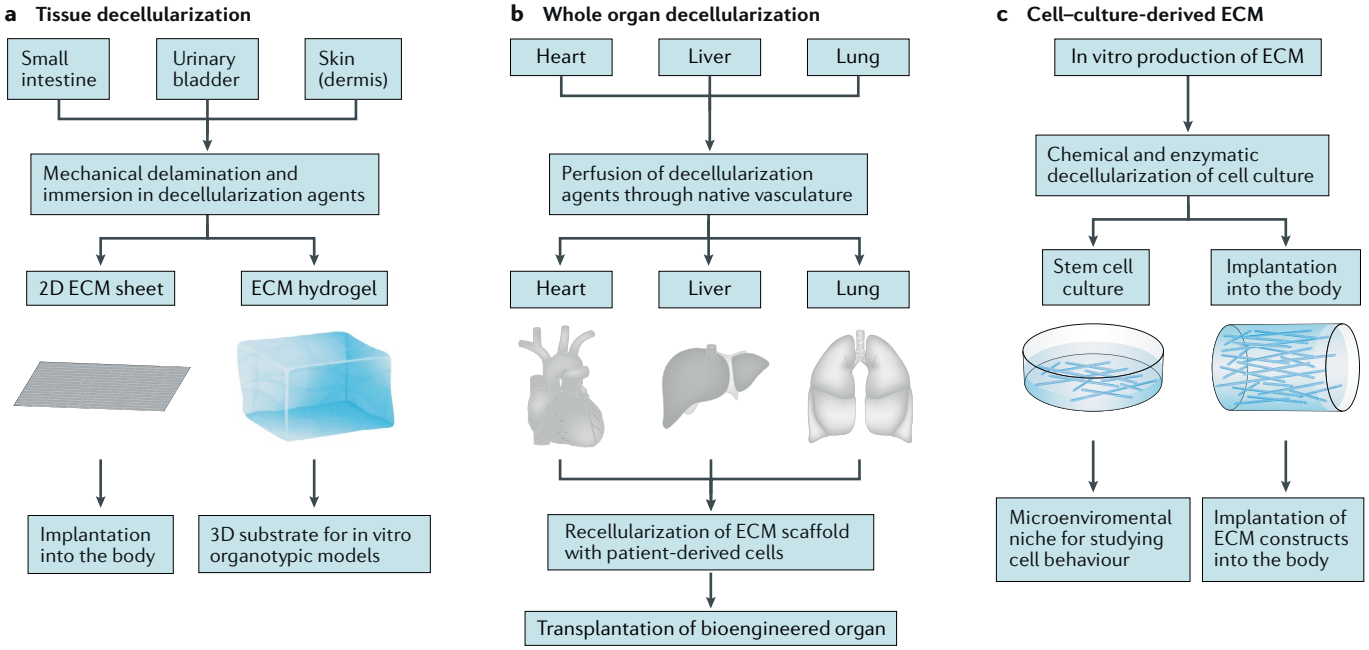

Fig. 4 | **Decellularization strategies. a** | Tissue can be decellularized to produce extracellular matrix (ECM) scaffolds for tissue regeneration. Mechanical delamination and chemical decellularization agents can be used to decellularize a variety of tissues, such as small intestine, urinary bladder and dermis, to create planar ECM sheets that can be further processed into ECM hydrogels. These substrates are used as surgical mesh or patch graft for implantation or as an in vitro organotypic model to study cell behaviour. **b** | Whole organs can be decellularized for the bioengineering of transplantable organs. Perfusion of decellularization agents through the native vasculature of organs such as the heart, liver and lung results in a 3D ECM scaffold that can be repopulated with patient-derived cells to engineer transplantable human organs. **c** | Isolated cells cultured in Petri dishes or in 3D synthetic templates produce ECM molecules in vitro, which can be harvested by decellularization techniques. Cell-culture-derived ECM constructs can be implanted into the body to repair damaged tissue, or they can be used as substrates to recreate a microenvironmental niche for the study of stem cell behaviour.

in scar tissue formation[185,212–215]. Standard criteria for tissue decellularization have not yet been officially established, but commonly accepted metrics for assessing decellularization include haematoxylin & eosin (H&E) and 4′,6-diamidino-2-phenylindole (DAPI) staining to verify the absence of cells and cell nuclei and to assess the reduction in the amount and base pair length of double-stranded genomic DNA[184,216].

Decellularized matrix for soft tissue repair. Decellularized ECM scaffolds were initially developed and regulated as surgical mesh materials and have been approved by the Food and Drug Administration (FDA) for a large number of clinical applications[217], including ventral hernia repair[218], musculoskeletal reconstruction[219], oesophageal reconstruction[220], dura mater replacement[221], breast reconstruction[222] and cardiac repair[223] (TABLE 1). Implantation of decellularized ECM scaffolds results in constructive remodelling[224], that is, an at least partial restoration of functional, site-appropriate tissue. Decellularized ECM scaffolds are typically xenogeneic and can be fabricated as single or multilaminate sheets for use as surgical mesh or patch graft (FIG. 4a). Alternatively, they can be designed as tubular grafts, powders or hydrogels[161]. Importantly, these xenogeneic scaffolds do not elicit an adverse innate or adaptive immune response[89,212]. Several factors determine the clinical outcome of ECM scaffold transplantation[44,214,215,225,226], including surgical technique, selected ECM scaffold for a specific clinical condition, age of the allogeneic or xenogeneic tissue donor and patient comorbidities. The clinical outcome is generally dictated by the host response to the ECM scaffold following implantation, including angiogenesis[216], innervation[227–229], stem cell recruitment[230,231], antimicrobial activity[227,232] and modulation of the innate immune response[233]. The major determinant of functional remodelling is the temporal host immune response to ECM scaffolds[88,233–237] and to their degradation products, which direct tissue repair through the anti-inflammatory M2-like macrophage and T helper 2 (T_H2) cell response associated with reduced local inflammation and constructive crosstalk with stem and progenitor cells[233,238] (FIG. 5).

Whole organ engineering. Decellularized ECM scaffolds can also serve as templates for whole organ engineering (FIG. 4b). Specifically, the development of perfusion decellularization methods and the subsequent recellularization with stem and progenitor cells have opened new possibilities for the replacement of damaged or diseased organs[239]. The basic strategy for the generation of transplantable human organs involves the decellularization of human or animal organs by venous perfusion with decellularization reagents, which results in a 3D scaffold with intact vasculature. Decellularized ECM scaffolds are then maintained in bioreactor systems designed

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Table 1 | **Clinical applications of decellularized extracellular matrix scaffolds for soft tissue repair**

Application	ECM source	Refs
Rotator cuff repair	Porcine SIS	288
	Human dermis	289
Oesophageal repair	Porcine SIS	220
Volumetric muscle loss	Porcine SIS	219
	• Porcine SIS • Porcine UBM • Porcine dermis	290
Peyronie disease	Porcine SIS	291
Facial reconstruction	Porcine SIS	292
Gingival recession	Human dermis	293
Breast reconstruction	• Fetal bovine dermis • Human dermis	294
Mitral valve replacement	Porcine SIS	295
Aortic valve replacement	Human pulmonary valve	296
Reconstruction of congenital heart defects	Porcine SIS	297
Cardiac patch	Porcine SIS	298
Ventral hernia repair	Porcine dermis	299
Vascular patch	Bovine pericardium	300
Diabetic ulcers	Porcine UBM	301
Closure of anorectal fistulas	Porcine SIS	302
	Porcine dermis	303
Dura mater repair	Porcine SIS	221
	Human dermis	304
Repair of critical-sized skin defects	Human dermis	305
	Porcine SIS	306
Pelvic floor reconstruction	Porcine dermis	307
	Porcine SIS	308

ECM, extracellular matrix; SIS, small intestinal submucosa; UBM, urinary bladder matrix.

to mimic the physiological conditions experienced by the specific organ (for example, electrical conduction, pressure gradients, pH, temperature and oxygen concentration)[240]. Perhaps the most challenging step is the recellularization of the ECM scaffold with appropriate cell types and numbers to match native cell distribution; important factors to consider are cell source and concentration, cell seeding routes and methods (for example, venous perfusion of cell mixtures or direct injection into the parenchyma) and bioreactor charateristics[241]. Preliminary studies using engineered organs have been performed in animal models, resulting in limited organ-specific functions[182], for example, as a result of orthotopic transplantation of recellularized kidney, lung, heart and liver. Despite recent progress in whole organ engineering, challenges remain for clinical translation, including optimization of decellularization and recellularization techniques, endothelialization of the vasculature and optimization of bioreactor systems[241].

In vitro model systems. Decellularized ECM has also been extensively used as a substrate for in vitro cell culture systems to maintain tissue-specific cell phenotypes[242,243], to induce chemotaxis of lineage-directed progenitor cells[244,245] and to modulate cell proliferation and differentiation[246–248] (FIG. 4a). For example, ECM derived from decellularized oesophageal mucosa or small intestinal submucosa promotes the formation of 3D organoids in vitro[158,249]. Decellularized liver ECM can serve as an in vitro model for the evaluation of drug metabolism and liver biology[250]. Moreover, physiological models based on decellularized ECM can be applied to study disease progression. For example, healthy perilesional-derived ECMs and colorectal cancer (CRC)-derived ECMs have different effects on the homeostasis, function and phenotype of epithelial cancer cells[251]. In addition, decellularized human colon tissue can be used as an organotypic model to study CRC progression by recellularizing the colon ECM with epithelial cells with oncogenic mutations[252]. Similarly, decellularization of tumour xenografts derived from A549 human pulmonary adenocarcinoma cells has been explored for models of 3D tumour microenvironments[253]. In vitro models of colon and liver cancer can also be used in pharmacological studies to evaluate the efficacy of anticancer drugs[254,255]. Such ECM-based in vitro models of the tumour microenvironment can provide information for the characterization of specific signalling

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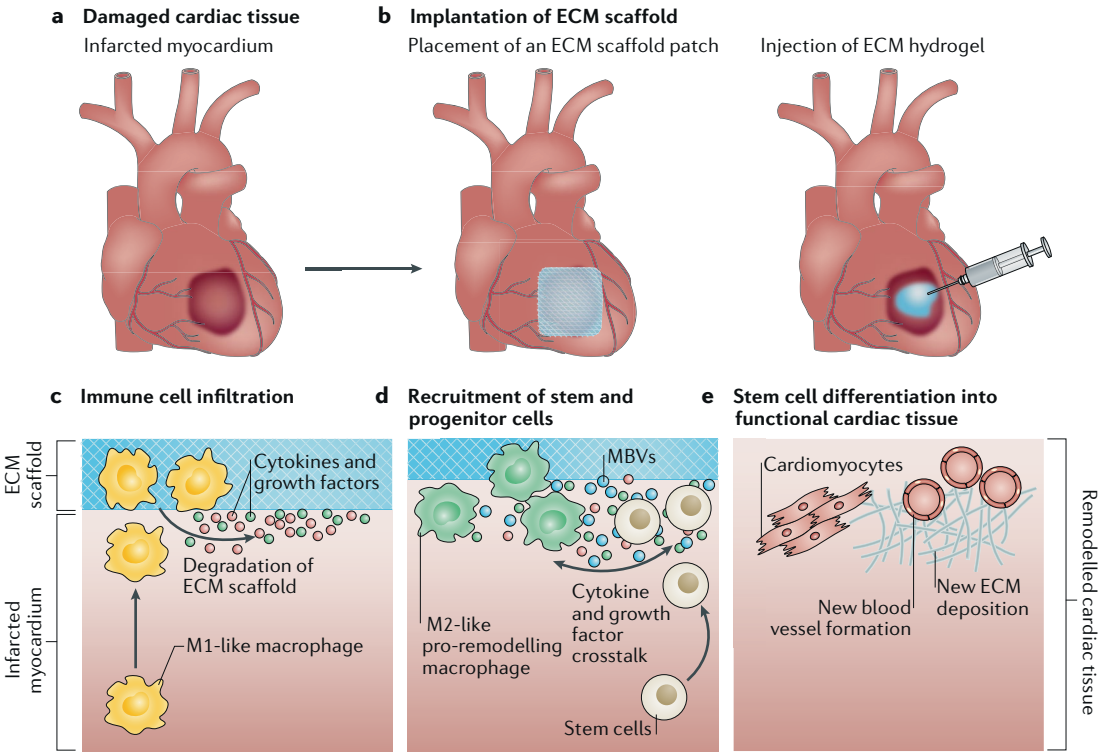

Fig. 5 | **Constructive remodelling by extracellular matrix biomaterials. a** | Extracellular matrix (ECM) scaffolds can be used to treat tissues that are damaged or diseased, for example, by myocardial infarction. Implantation of ECM scaffolds relies on the body as a natural bioreactor and the recruitment of endogenous stem cells. **b** | ECM scaffolds can be fabricated as patch grafts that can be placed on damaged tissue areas or as hydrogels that can be directly injected into the tissue. **c** | Upon implantation of the ECM biomaterial, immune cells infiltrate the material, resulting in the activation of a pro-inflammatory M1-like macrophage phenotype. **d** | Following the initial host immune response, the ECM scaffold undergoes rapid degradation, releasing bioactive growth factors, cryptic peptides and matrix-bound nanovesicles (MBVs) that mediate the activation of a pro-remodelling M2-like macrophage phenotype and the recruitment of endogenous stem and progenitor cells. **e** | The coordinated interplay between infiltrating immune cells and stem and progenitor cells, including the establishment of autocrine and paracrine signalling networks, promotes stem cell differentiation, angiogenesis and the deposition and remodelling of new ECM components, resulting in the formation of site-specific, functional host tissue.

molecules involved in cancer progression and can serve as screening tools for cancer therapeutics.

Cell-culture-derived matrix. Cultured cells produce ECM in vitro, which can be isolated using similar decellularization protocols as for whole tissues[256] (FIG. 4c). Using this approach, autologous cells can be cultured to obtain specific ECMs, obviating concerns regarding xenogeneic or antigenic cell types. Moreover, ECM geometry and porosity can be readily modulated, and the ECM can be generated and maintained in a pathogen-free environment[256,257]. In addition, cell-culture-derived ECM constructs can be customized by controlling the cell culture environment, for example, through modulating oxygen concentration[258] and mechanical preconditioning[259]. Furthermore, cell-derived ECMs can be produced in various 3D shapes and architectures using synthetic polymers as moulds[260,261] and can be equipped with additional functionalities. For example, human dermal fibroblast-derived ECMs can be modified with sterically accessible azide-modified sugar analogues using bio-orthogonal click chemistry to covalently immobilize the ECM on an artificial surface to provide a stable ECM coating for biomaterials[262]. Cell-culture-derived ECM substrates can also be used as stem cell niche models to study cell behaviour[263,264]. Moreover, culturing cancer cells in a realistic pathophysiological ECM provides a useful tool for the investigation and development of cancer therapies[265,266]. Cell-culture-derived ECM substrates used for the generation of blood vessels and heart valve constructs have shown limited success in preclinical[267–269] and clinical studies[270–272] thus far. However, the use of cell-culture-derived ECM substrates for biomedical applications is still in its infancy, and benefits, such as faster, easier and cheaper generation of cell-derived ECMs than with tissue-derived approaches, should not be overlooked in terms of translational efficacy[273].

Conclusions

The native mammalian ECM constitutes an ideal microenvironmental niche for functional tissue reconstruction. The ECM contains intrinsic biochemical and mechanical cues that regulate cell phenotype and function in development, in homeostasis and in response to injury. Although the past few decades have witnessed substantial progress in the development and use of ECM biomaterials, major hurdles remain for the widespread

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Box 1 | **Major objectives for the clinical translation of extracellular matrix biomaterials**

- Improved imaging and data analysis techniques to conduct a detailed mapping of the native extracellular matrix (ECM) topography for accurate reproduction of topological features.
- Improved methods and metrics for tissue and organ decellularization to avoid a pro-inflammatory immune response following implantation.
- Identification of specific ECM-derived molecular cues that modulate the innate and adaptive immune response.
- Continued development of the matrisome, including analysis of nonfibrillar components, such as lipids and nucleic acids, that are functionally integrated into the matrix, and detailed investigation of the spatial and temporal changes in the ECM composition in development, homeostasis and ageing, as well as in response to injury.
- Understanding of mechanical loading as a determinant of constructive and functional remodelling.
- Identification of specific ECM-derived molecular cues that activate quiescent tissue-resident stem and progenitor cells and characterization of the temporal ECM-initiated and ECM-mediated crosstalk between macrophages and stem cells that populate the ECM scaffold during degradation and remodelling.
- Identification of the genomic and epigenetic signals that influence ECM composition in development, in disease and in response to injury.

clinical translation of ECM biomaterials (BOX 1). Proteomic and bioinformatics techniques have begun to provide a more rigorous mapping of ECM components in various tissues in healthy and pathological states, enabling a detailed investigation of ECM molecules and their integration into biomaterials. However, a greater understanding of the biochemical and structural components that constitute native ECMs is required to design and fabricate ECM biomaterials that meet the anatomical and physiological needs of native tissues. Simplified bottom-up approaches allow the spatial arrangement of ECM components during the fabrication processes; however, these synthetic ECM substrates do not recreate the complex ultrastructure and composition of the native matrix. Furthermore, major gaps in our understanding of ECM dynamics, such as proteolytic degradation during matrix turnover, the release of cryptic peptides, the timing of signals and the modulation of their spatial arrangement, limit our ability to design and fabricate ECM biomaterials that fully mimic the tissue-specific and state-specific biochemistry and architecture of native mammalian ECM. Alternatively, a top-down approach using decellularization of native tissues and organs has advanced the applications of ECM biomaterials and enabled the use of ECM biomaterials for clinical applications to promote in situ tissue remodelling and as an ex vivo template for whole organ engineering. Progress in decellularization techniques and optimization of recellularization strategies will improve ECM scaffold biocompatibility, endothelialization and functional anastomosis into the host vasculature. To achieve these goals, a multidisciplinary approach is required, integrating principles of cell biology and materials science. Our understanding of the ECM continuously evolves, and technology is constantly improved; thus, the future of ECM biomaterials in tissue engineering and regenerative medicine applications is promising.

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G.S.H., J.L.D. and S.F.B. researched data for the article and made substantial contributions to discussion of content. G.S.H., J.L.D. and S.F.B. wrote the article and reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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