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REVIEW ARTICLE

Engineering Stem Cell-Derived Extracellular Matrices: Decellularization, Characterization, and Biological Function

Sébastien Sart, PhD,[1,2] Richard Jeske, BS,[3] Xingchi Chen, MS,[3] Teng Ma, PhD,[3] and Yan Li, PhD[3]

Stem cells, including mesenchymal stem cells and pluripotent stem cells, have attracted considerable attention in tissue engineering and regenerative medicine primarily because of their unique ability in self-renewal and multilineage differentiation. However, stem cells also have important secretory functions that form a specialized *in vivo* microenvironment and direct tissue development and regeneration. Extracellular matrices (ECMs) derived from stem cells retain the functional properties of their native environment and exhibit unique signaling that mediates stem cell self-renewal and lineage commitment. Stem cell-derived ECMs (scECMs) also have tunable properties corresponding to their developmental stages, suggesting that their lineage- and developmental specificity can be engineered for a wide range of applications. Hence, there is a growing interest in reconstructing stem cell microenvironment through decellularization and obtaining decellularized matrices that exhibit unique biological properties. This article summarizes recent advances in the use and understanding of scECMs. Moreover, future directions to extend the spectrum of applications of stem-derived ECMs in tissue engineering by comprehensively elucidating and engineering their regulatory function is highlighted.

Keywords: extracellular matrix, pluripotent stem cells, mesenchymal stem cells, decellularization, secretome

Impact Statement

Stem cells bear unique potency for multilineage differentiation as well as the capacity to secrete a vast amount of regulatory molecules. At different developmental stages, the extracellular matrices (ECMs) secreted by stem cells regulate their microenvironment and direct tissue development. The decellularization of stem cells effectively preserves ECM functional properties and can provide suitable templates to regulate stem cell fate decision, which can hardly be reproduced using single ECM proteins or synthetic scaffolds. This review highlights the unique regulatory functions of stem cell-derived ECMs, which can serve as novel sources of highly bioactive materials for tissue engineering and cell therapy.

Introduction

In recent years, stem cells, including adult mesenchymal stem cells (MSCs) and pluripotent stem cells (PSCs), have emerged as promising tools for tissue engineering, drug screening, and disease modeling.[1–3] MSCs, derived from connective tissues such as bone marrow stroma, adipose tissues, and umbilical cords, have differentiation potentials along adipogenic, osteogenic, and chondrogenic lineages.[4] MSCs also have broad trophic activities, including immune regulatory properties, the secretion of various growth factors that can stimulate the differentiation of endogenous progenitors in tissues, and proangiogenic function.[5] Due to these unique properties, MSCs have been tested in more than 1000 clinical trials (www.clinicaltrials.gov) to treat a variety of diseases.

Different from MSCs, PSCs, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have extensive self-renewal ability and lineage-specific differentiation potential into cells from all the three germ layers.[6] Due to the ability to recapitulate embryonic development, PSCs provide a unique platform for drug screening, pathological disease modeling, and stem cell therapy.[7]

Both MSCs and PSCs share two defining properties of stem cells: the ability to self-renewal and to differentiate into specific lineages, which enable MSCs to maintain

[1]Hydrodynamics Laboratory, CNRS UMR7646, Ecole Polytechnique, Palaiseau, France.
[2]Laboratory of Physical Microfluidics and Bioengineering, Department of Genome and Genetics, Institut Pasteur, Paris, France.
[3]Department of Chemical and Biomedical Engineering, FAMU-FSU College of Engineering, Florida State University, Tallahassee, Florida, USA.

normal mesenchymal tissue turnover and for PSCs to generate all tissue types.[8,9] These processes are in a large part sustained by the interactions between stem cells and their extracellular microenvironments.[10]

In vivo, MSCs in the bone marrow interact with a complex extracellular matrix (ECM) network, which can remodel with time and release endogenous and exogenous signaling molecules to support hematopoietic stem cells and induce lineage-specific differentiation.[11,12] Similarly, PSC proliferation and differentiation recapitulate embryonic tissue development and are accompanied by dynamic remodeling of ECMs and local gradients of morphogens.[13–15] Hence, ECM proteins and regulatory factors surrounding stem cells form a regulatory niche and play essential roles in modulating stem cell fate.[16,17] Understanding and recapitulating the characteristics of stem cell microenvironment should significantly improve our ability to control stem cell fate in tissue regeneration and cell therapy.

In culture, both MSCs and PSCs are able to secrete a large amount of endogenous ECMs, which reflect their developmental stages[18–20] and the characteristics of their *in vivo* microenvironments.[21,22] Decellularization of tissues or cultured cells can effectively preserve intact ECM proteins and the bound regulatory growth factors while removing cellular components (DNA, lipids, etc.) that could lead to immunogenicity *in vivo* (Fig. 1).[23–25] The stem cell-derived ECMs (scECM) can be readily obtained from cultured stem cells through decellularization and have been shown to contain a broad spectrum of paracrine and autocrine factors.[14,16,24,26] These acellular matrices have been shown to retain specific signaling cues and structural features that direct stem cell fate *in vitro* and tissue regeneration *in vivo*.[12,19,27–31] Decellularized ECMs have also been used as coating agents or directly as 3D scaffolds to construct functional tissues.[19,32–34] This review investigates the roles of decellularized matrices derived from MSCs and PSCs in modulating stem cell fate decisions. Specifically, recent advances are summarized to understand the specific molecules and signaling of decellularized matrices toward a better utilization of scECMs as versatile bioactive scaffolds in tissue engineering and regeneration.

Stem Cell-Derived Extracellular Matrices and Their Role in Directing Stem Cell Fate Decisions

In vivo, stem cells reside in specialized niches that are known to regulate stem cell fate throughout their life span.[12] Among various niche factors, such as cytokines, cell–cell contacts, and adhesion molecules, ECMs play a central role in orchestrating endogenous and exogenous signals. The ECM network not only provides structural support but also regulates stem cell behaviors through biochemical composition, sequestration of bioactive factors, and presentation of biomechanical cues.[16,35]

The biochemical composition of ECMs regulates stem cell proliferation and differentiation through EC–integrin interactions (Fig. 1A). Indeed, to sustain self-renewal, human PSCs (hPSCs) are usually grown on Matrigel (the main component is laminin) or vitronectin through binding with $\alpha6\beta1$ or $\alpha v\beta5$ integrins, respectively.[36,37] Similarly, a combination of collagen I, collagen IV, fibronectin, and laminin or vitronectin was reported to promote the propagation of human ESCs (hESCs) at the undifferentiated state.[38] Mouse ESCs remain at an undifferentiated state on collagen type I and type IV or poly-D-lysine, while they spontaneously differentiate on laminin and fibronectin due to different ECM–integrin interactions.[39] Conversely, neural differentiation of hESCs was shown to be promoted on laminin, while a mixture of fibronectin and vitronectin favored endodermal commitment and collagen type IV-induced mesodermal differentiation.[13,40,41] For MSCs, cell proliferation was differentially regulated on collagen type IV, vitronectin, fibronectin, and laminin that is mediated by multiple $\beta1$ integrins or $\alpha v\beta3$ integrin for vitronectin.[42,43] In addition, osteogenic differentiation of MSCs was found to be promoted on collagen type I and vitronectin. However,

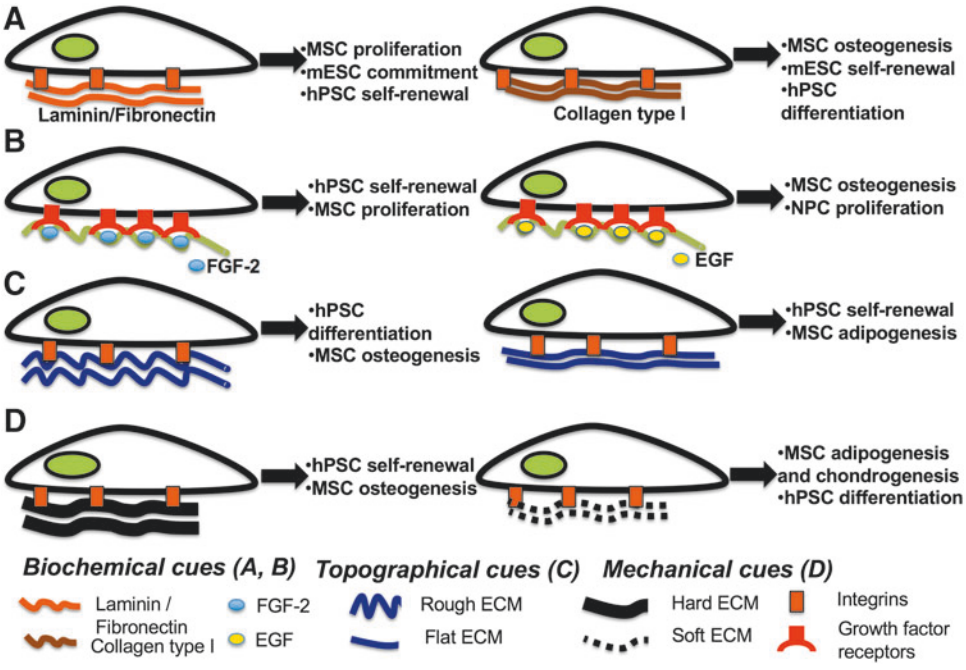

FIG. 1. Regulation of stem cell fate decisions by ECM cues. **(A)** Regulation of stem cell proliferation and differentiation by the biochemical composition; **(B)** the immobilized growth factors; **(C)** the topography; and **(D)** the mechanical properties of the ECM. ECM, extracellular matrix; EGF, epidermal growth factor; FGF-2, fibroblast growth factor 2; hPSC, human pluripotent stem cell; mESC, mouse embryonic stem cells; MSC, mesenchymal stem cell; NPC, neural progenitor cell. Color images are available online.

the denatured form of collagen I favors adipogenic differentiation due to integrin-mediated signaling,[42,44] while chondrogenic differentiation is promoted on collagen type II and chondroitin sulfate.[45]

ECMs not only support cell adhesion through integrin binding but also enhance the stability of growth factors by providing the binding sites that can regulate their release and prolong biological potency (Fig. 1B).[18,46] Binding with ECMs also potentiates the effects of growth factors compared with their unbounded counterparts. The enhancement of growth factor activity through ECM binding has been observed for hepatocyte growth factor (HGF), bone morphogenetic protein (BMP)-2 and -4, and acidic fibroblast growth factor (FGF) during hESC hepatic differentiation.[47] Our prior study evaluated matrix-bound FGF-2 and the results show that ECMs decellularized from human MSCs retain their binding capacity to both exogenous and endogenous FGF-2.[18] Similarly, the release of endogenous BMP-2 and insulin-like growth factor (IGF)-1 from decellularized matrix of bone marrow and adipose tissue-derived MSCs was demonstrated to enhance osteogenic differentiation of reseeded MSCs.[46]

The topography of the ECMs is also a potent regulator of stem cell fate (Fig. 1C, D). The nanostructure of the substrates was shown to affect the binding of ECM proteins such as fibronectin and laminin due to local curvature, which influences the adsorption of ECM proteins.[48,49] In addition, osteogenesis of MSCs was enhanced on rough surfaces while smooth surfaces favored adipogenesis of MSCs. Similarly, the self-renewal of hESCs was promoted on smooth surfaces (with roughness of 1 nm) whereas rough surfaces (with roughness of 150 nm) led to spontaneous differentiation through signal transduction through E-cadherin-mediated cell–cell interactions.[50,51] These observations indicate that mimicking the nanostructure of cellular microenvironment may be able to predict the stem cell fate.

Recently, the mechanical property of ECMs was found to play a critical role in lineage commitment of stem cells.[52] The increased matrix stiffness (e.g., 34 kPa) was shown to enhance osteogenic differentiation of MSCs, while soft substrates (e.g., 1 kPa) favored chondrogenesis or adipogenesis.[53–55] This indicates that MSC differentiation due to mechanical cues (i.e., elastic modulus) primarily corresponds to *in vivo* localization of the cells. The induced osteogenesis of MSCs on stiff ECMs may mimic what occurs in the bone marrow. For mouse ESCs, soft matrices can support self-renewal by generating low cell–matrix tractions and low cellular stiffness, while hard substrates can induce the differentiation toward mesodermal and endodermal lineages.[56,57] In contrast, hESC propagation at undifferentiated state was promoted on stiff substrates by activation of paralogous proteins, Yes-associated protein (YAP)–transcriptional coactivator with a PDZ-binding domain (TAZ), while mesodermal, endodermal, and ectodermal differentiations were differentially induced in an elastic modulus-dependent manner.[58,59] YAP–TAZ has been recognized recently as a potent regulator for mechanotransduction signaling in stem cells.[60,61]

Different from simple ECM mixtures, the scECMs have specific functional and signaling capacity that cannot be readily reproduced using single ECM proteins or synthetic matrices.[14,25,62] The dynamic ECM remodeling and the sequestration and release kinetics of ECM–growth factor interactions actively coordinate the signaling cascade during stem cell self-renewal and lineage commitment, providing a broader spectrum of signaling specificity corresponding to the specific tissue development stage that is absent in ECMs derived from somatic cells.[12,27,29,46,63] These properties provide the opportunity to tailor scECM properties for specific *in vitro* and *in vivo* applications. For example, transplantation of MSC-derived ECMs was reported to enhance bone repair *in vivo* by extending site-specific human MSC retention, and substantially improved clinical outcome.[25,64] hPSC-derived ECMs have also been shown to contain growth factors acting on the transforming growth factor (TGF)-β/Nodal pathways, which can support hPSC propagation and suppress tumor phenotype.[62,65] The dynamic interactions of scECMs with paracrine and autocrine growth factors constitute unique microenvironments to regulate stem cell fate decisions.

Derivation and Characterization of scECMs

Decellularization methods

Preserving the biochemical, biological, and biophysical properties of scECM is a focus of current ECM research, and various decellularization methods developed for somatic tissues and organs have been tested for stem cell cultures (Table 1).[23] Among these methods, mechanical disruption and chemical agent treatments have been mostly used to remove cellular materials while retaining signaling molecules and structural properties.

Mechanical decellularization methods include freeze drying through lyophilization, sonication, or pressurization.[23,66,67] Freeze-drying cycles were reported to disrupt cell membrane and release intracellular materials (e.g., DNA, organelles) of PSC-derived embryoid bodies (EBs).[66] This method also demonstrates the partial retention of ECM proteins such as collagen and glycosaminoglycans (GAGs) as well as ECM-bound growth factors, such as TGF-β1, IGF-1, and vascular endothelial growth factor (VEGF).[68] However, freeze drying, sonication, or pressurization may alter the ECM protein structure (such as collagen fibers) and the mechanical properties of ECMs.[67,69]

Chemical methods use agents such as Triton X-100, sodium deoxycholate (SDC), and sodium dodecyl sulfate (SDS) to remove cellular materials (Table 1). Triton X-100 can break DNA–protein, lipid–lipid, and lipid–protein connections in various stem cell organizations.[19,23,70] Studies have shown that the decellularization by Triton X-100 does not alter ECM structure and that the decellularized ECMs retain their capacity to bind and maintain the bioactivity of growth factors. For example, Triton X-100 does not change laminin self-assembly,[71] the binding of fibronectin to gelatin and heparin,[72] or the heparin-binding affinity of vitronectin.[73,74] This nonionic detergent also enables the retention of platelet-derived growth factor (PDGF), BMP-4, epidermal growth factor (EGF), FGF-2, HGF, and VEGF, and preserves the bioactivity of the bound growth factors such as FGF-2.[75–77] However, Triton X-100 may denature GAG content and affect collagen fiber structure.[78] Both SDS and SDC can solubilize the cell and nucleic membranes. But SDS tends to denature the ECM proteins such as fibronectin and decrease the factor-binding affinities to the ECMs such

Table 1. Summary of the Different Decellularization Methods (Advantages and Disadvantages)

Decellularization method	*Effect on DNA removal*	*Effect on ECM protein retention*	*Effect on ultrastructure, topography, and mechanical properties*	*Effect on growth factor retention in ECMs*	*Effect on GAG retention*	*Ref.*
Mechanical methods						
Freeze drying	Efficient DNA removal	Partial retention of ECM proteins (collagens)	Alteration of protein structure and ECM mechanical properties	Partial retention of growth factors, for example, TGF-β1, IGF-1, and VEGF	Partial retention of GAGs	Badylak *et al.*[23,161] Ngangan and McDevitt[66] Hirata and Yamaoka[67]
Sonication	Efficient DNA removal	Partial retention of ECM proteins (collagens)	Alteration of protein structure and mechanical properties	N.D.	Denaturation of GAGs	Azhim *et al.*[162]
Hydrostatic pressure	Efficient DNA removal	Partial retention of ECM proteins (collagens)	Retained structure and mechanical properties	N.D.	Retention of GAGs	Wu *et al.*[163] Hashimoto *et al.*[164] Watanabe *et al.*[165]
Chemical methods						
SDS	Efficient DNA removal	Partial retention of ECM proteins (collagens)	Denaturation of ECM proteins	Limited retention of growth factors	Denaturation of GAGs	He *et al.*[166] Haas and Culp[72]
SDC	Efficient DNA removal	Limited retention of ECM proteins	Denaturation of ECM proteins	Limited retention of growth factors	Denaturation of GAGs	Fischer *et al.*[167]
Triton X-100	Efficient DNA removal	Partial retention of ECM proteins (collagens)	Partial preservation of ECM structure	Retention of growth factors, for example, PDGF, BMP-4, EGF, FGF-2, HGF, and VEGF	Denaturation of GAGs	Freire and Coelho-Sampaio[71] Haas and Culp[72] Barnes *et al.*[73] Peterson[74] Wolf *et al.*[75] Brown *et al.*[76] Chun *et al.*[77] Liao *et al.*[78]
Calcium hydroxide	Efficient DNA removal	Limited retention of ECM proteins	Alteration of ECM mechanical properties	Limited retention of growth factors	Denaturation of GAGs	Badylak [161] Sengyoku *et al.*[168]
Sodium sulphide	Efficient DNA removal	Limited retention of ECM proteins	Alteration of ECM mechanical properties	Limited retention of growth factors	Denaturation of GAGs	Badylak [161] Crapo *et al.*[80]
Sodium hydroxide	Efficient DNA removal	Limited retention of ECM proteins	Alteration of ECM mechanical properties	Limited retention of growth factors	Denaturation of GAGs	Badylak [161] Crapo *et al.*[80]

BMP, bone morphogenetic protein; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; GAGs, glycosaminoglycans; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; N.D., not determined; PDGF, platelet-derived growth factor; SDC, sodium deoxycholate; SDS, sodium dodecyl sulfate; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

as FGF-2, VEGF, and TGF-β1.[72] SDC was reported to be more disruptive than SDS, leading to the degradation of ECM structure and GAGs, limiting the growth factor retention.[76] Bases such as calcium hydroxide, sodium sulfide, and sodium hydroxide can solubilize cytoplasmic components and disrupt nucleic acids.[23] However, bases are reported to partially remove the growth factors and alter the ECM mechanical properties. Alternatively, DNAses and RNAses are able to cleave cellular nucleic acids without affecting the protein content of ECMs and have been commonly used in deriving scECMs.[79] Altogether, mechanical and chemical decellularization methods can efficiently remove cellular materials. However, the retention of ECM proteins, growth factors, and mechanical properties is highly variable among various methods and cell or tissue sources, and thus need to be evaluated for particular applications. Taken together, while mechanical methods are preferred for the decellularization of large tissues, chemical treatments (e.g., Triton X-100) that diffuse more efficiently into small stem cell aggregates or monolayers may be more effective to preserve regulatory function of native scECMs (Table 1).

Decellularized ECM characterizations

To ensure the efficiency of decellularization processes, scECMs are usually characterized by the absence of remaining cellular DNA, which is achieved by direct staining and quantitative imaging using base-intercalating molecules (e.g., Picgreen) after standard DNA isolation procedures (e.g., phenol/chloroform).[80] The ultrastructure of scECMs are usually assessed using scanning electron microscopy, and the mechanical properties can be measured using atomic force microscopy or dynamic mechanical analysis by applying a sinusoidal stress.[20]

Cell-derived ECMs are undefined materials containing numerous proteins and their characterizations provide important information to elucidate the signaling molecules. While enzyme-linked immunosorbent assays and immunocytochemistry are usually used to quantify a restricted number of proteins, the critical components of acellular ECMs can be identified using proteomics analysis based on mass spectrometry (MS) to delineate the shared and distinct components in ECMs at different stages of stem cell development.[29,81–84] To identify the critical components of acellular ECMs, proteomic analysis based on MS has been used to delineate the shared and distinct components in ECMs at different stages of stem cell development.[29,81–84] However, efficient solubilization of the ECM compounds, the retention of their integrity, and accurate characterizations of their complexity remain a critical challenge to date.[29,82] Decellularized ECMs from MSCs undergoing osteogenic differentiation (osteoECM) and MSCs treated with ascorbic acid (aaECM) were analyzed by MS.[12] Up to 45% of proteins were annotated by Gene Ontology as ECM proteins, and 26% had molecular functions of ECM structural constitutes. aaECM was found to contain twice as much collagen and GAGs compared with osteoECM. The ECMs were also able to release the immobilized growth factors, including HGF, FGF, VEGF, and interleukin (IL)-8 especially for aaECM.[12] The ECMs produced by MSCs overexpressing active component of Notch signaling (i.e., NICD) were found to secrete a higher amount of fibronectin, fibrilin, febulin, and several collagen subtypes and exhibit the enhanced neuroprotective function.[82] Proteomic analysis was also performed to compare functional patterns of MSC-derived ECMs from different donors and tissue sources, such as bone marrow, amniotic fluid, or adipose tissue.[29,85] Consistent ECM profiles were observed among different donors, demonstrating the possibility of off-the-shell ECM production.[29]

For PSCs, proteomic analysis has been performed to elucidate the ECM proteins and growth factors that are involved in hPSC self-renewal and differentiation. The ECMs derived from the feeder fibroblasts were characterized to identify the proteins (e.g., heparin sulfate proteoglycan) responsible for hPSC propagation.[86] The undifferentiated hPSCs were found to deposit complex ECMs with a 57% overlap with the ECMs secreted by the supportive fibroblast feeders.[87] Our study compared the proteomics of ECMs derived from undifferentiated PSC aggregates (AGG), spontaneous embryoid bodies (EBs), and aggregates of PSC-derived neural progenitor cells (NPCs). NPC-ECMs are more distinctly different (27% of individually expressed proteins) compared with AGG-ECMs (16%) and EB-ECMs (12%).[84] All these studies demonstrate distinct ECM microenvironment are generated at different stages of PSC development. Moreover, recent proteomic analysis reveals significant changes in the structure of proteoglycans produced upon differentiation of hESCs toward endoderm and mesoderm lineage.[88]

Regulation of Stem Cell Fates on Tissue-Specific Acellular Matrices

scECM studies are largely based on the knowledge learned from tissue-specific acellular matrices (tsECMs). In addition, the plasticity of MSCs and PSCs can be demonstrated by culturing on tsECMs. Moreover, to show the differences between scECMs and tsECMs, it is necessary to discuss the work done for tsECMs first.

Mesenchymal stem cell proliferation and differentiation on tsECMs

Decellularized matrices derived from somatic tissues retain the cues of original tissue sources and can direct the lineage-specific differentiation of MSCs (Table 2). Indeed, MSCs seeded on decellularized bone matrix have higher differentiation efficiency toward osteogenic lineage compared with cells on tendon-derived matrix.[89] ECMs derived from adipose tissue or cartilage were also shown to induce MSC differentiation into adipocytes or chondrocytes, respectively.[90–93] However, the molecular mechanisms involved in the modulation of MSC behaviors on a particular decellularized matrix are still not well understood. For various tsECMs, collagen type I is the most characterized ECM protein preserved after decellularization and its abundance may contributes to the increased MSC proliferation on acellular ECMs.[42] The direct interactions between ECM proteins of the decellularized matrices (e.g., collagen) and specific integrins (e.g., α2β1) of MSCs have been shown to regulate extracellular signal-regulated kinases (ERK)1/2 signaling and promote osteogenic differentiation.[94] Besides collagen I, most decellularized matrices used for MSC culture show the retention of GAGs, which preserve binding sites for adhesion molecules (e.g., fibronectin and vitronectin) and heparin-binding growth factors, such as

Table 2. Mesenchymal Stem Cell Expansion and Differentiation on Decellularized Matrix Derived from Tissues or Cultured Somatic Cells

ECM source	*MSC source*	*Decellularization method*	*Properties of the acellular matrix*	*Effect on MSC proliferation*	*Effect on MSC differentiation*	*Ref.*
Tissues						
Bone marrow	Tendon	Tendon ECM: Physical/chemical treatment: freeze/thaw +DNAse	Preserved collagen	Fivefold increase	Tendinous for tendon-derived ECM (SCX, EYA2); bone-derived ECM: osteogenic differentiation (Runx2, Osteocalcin)	Yin *et al.*[89]
Tendon derived		Bone marrow ECM: Chemical treatment: NaOH, SDS				
Tendons	Adipose tissue	Physical/chemical treatment: Freeze dry, DNAse	Preservation of collagen	Support proliferation	Tenogenic differentiation (SCX, TNMD, TNC expression)	Yang *et al.*[169]
Adipose tissue	Adipose tissue	Physical/chemical treatment: Freeze/thaw, NaCl, trypsin/EDTA, Triton X-100	Preserved collagen, GAG, VEGF	Support proliferation	Support adipogenic differentiation (Oil Red O staining)	Wang *et al.*[170]
Adipose tissue	Adipose tissue	Physical/chemical treatment: α-amylase, lyophilization	Preservation of collagen	N.D.	Support adipogenic differentiation (GPDH, PPAR-γ, lipoprotein lipase expression)	Yu *et al.*[90]
Placenta	Adipose tissue	Chemical treatment: Lauroyl sarcosinate, DNase, RNase, Triton X-100	Preservation of collagen and elastin; Partial degradation of collagen IV and laminin	Support adhesion and proliferation	Adipogenic differentiation and vascularization (Oil Red O staining)	Flynn *et al.*[171]
Trachea	Adipose tissue	Physical/chemical treatment: povidone-iodine, trypsin-EDTA, Guanidine, SDC, SDS, tributyl phosphate, freeze/thaw	Absence of residual MHCs, retained collagen II and proteoglycans, retained thermal stability and elasticity	N.D.	Chondrogenic differentiation (SOX-9 expression)	Giraldo-Gomez *et al.*[172]
Annulus fibrosus	Annulus fibrosus	Chemical treatment: trypsin, ribozyme, Triton X-100, acetic acide. Crosslinking with genipin, chitosan, and FGF-2 incorporation	N.D.	Increased proliferation (1.5-fold)	Increased ECM expression (collagen type I and II, aggrecan)	Liu *et al.*[173]
Dental pulp	Bone marrow	Physical/chemical treatment: Trypsin-EDTA, SDS, DNAse, lyophilization, NHS, EDC crosslinking	Retained structural integrity and water absorption	Increased proliferation	Increased expression of collagen type I, DMP-1, and DSPP	Bakhtiar *et al.*[174]
Skin	Bone marrow	Physical/chemical treatment: Trypsin, SDS, EDTA, Triton X-100, isopropanol, lyophilization	Retained sGAG, collagens, α-elastin, VEGF	Retained proliferation, increased metabolic activity	N.D.	Ventura *et al.*[175]

(*continued*)

TABLE 2. (CONTINUED)

ECM source	*MSC source*	*Decellularization method*	*Properties of the acellular matrix*	*Effect on MSC proliferation*	*Effect on MSC differentiation*	*Ref.*
Adipose tissue	Adipose tissue	Physical/chemical treatment: freeze/thaw, trypsin, benzonase, isopropanol	Increased fibronectin and laminin expression postexternal volume expansion	Increased proliferation (twofold)	Increased adipogenic differentiation (perilipin, PPAR-γ, and CEBP-α)	Zhang *et al.*[176]
Heart	Adipose tissue	Chemical treatment: SDS, Triton X-100, pepsin-HCl solutions	Retained GAGs and ECM structure, collagen type I and III, fibronectin, laminin	Increased proliferation	Increased expression of cTnT and Cx43, and calcium transients	Bai *et al.*[177]
Skeletal muscle	Bone marrow	Chemical treatment: Triton X-100, NH_4OH, acetic acid	N.D.	Increased proliferation	Increased expression of myogenic proteins (myoD, myogenin, desmin)	Talovic *et al.*[178]
Abdominal skin	Bone marrow	Physical/chemical treatment: Freeze dry, DNAse	Preservation of collagens, no change in the mechanical properties	No difference from controls	Increased angiogenesis (CD31 expression)	Zhao *et al.*[97]
Bone	Bone marrow	Physical/chemical treatment: Hydrostatic pressure	Preservation of collagens	Increased proliferation	Increased osteogenic differentiation (threefold higher ALP)	Hashimoto *et al.*[164]
Cartilage	Bone marrow	Chemical treatment: EDTA, Triton X-100, DNAse, RNAse	Retained GAGs and collagen type II	Support proliferation	Chondrogenic differentiation (Safranin O, Tuoluidine Blue, and collagen II)	Yang *et al.*[179]
Cultured cells						
Fibroblasts (WI-38)	Adipose tissue	Chemical treatment: Triton X-100, NH_4OH	N.D.	Reduced proliferation Delayed senescence	Increased chondrogenic differentiation (Sox-9, Notch1, *p*-TGF-βRII, and Jagged1, collagen type II)	Dzobo *et al.*[180]
Osteoblasts (MC3T3-E1)	Bone marrow	Physical/chemical treatment: Triton X-100, Freeze/thaw, NH_4OH	Preserved GAG, collagen, and fibronectin	Increased proliferation (twofold)	Increased mineralization (Alizarin Red and o-cresolphthalein complexone staining)	Li *et al.*[92]
Primary fibroblasts, osteoblasts and chondro-cytes	Bone marrow	Physical/chemical treatment: Na_2HPO_4, NP-40	Retained structure, GAGs and collagen type II (chondrocyte-derived dECM), fibronectin (fibroblast-derived dECM)	N.D.	Increased chondrogenic differentiation on chondrocyte-dECM (collagen II and SOX-9). Increased collagen I on fibroblast-dECM; Increased osteogenic differentiation on osteoblast-dECM (ALP activity)	Mao *et al.*[93]

ALP, alkaline phosphatase; CBFA1, core-binding factor alpha 1; dECM, decellularized extracellular matrix; DMP-1, dentin matrix acidic phosphoprotein 1; DSPP, dentin sialophosphoprotein; EYA2, eyes absent homolog 2; GPDH, glycerol-3-phosphate dehydrogenase; MHC, major histocompatibility complex; MSC, mesenchymal stem cell; PPAR-γ, peroxisome proliferator-activated receptor-γ; Runx2, runt-related transcription factor 2; SCX, scleraxis; SOX-9, sex-determining region Y-box 9; TNC, tenascin C; TNMD, tenomodulin.

FGF-2, PDGF, VEGF (binds perlecan, heparin, dermatan sulfate, chondroitin sulfate, etc.), and TGF-β (binds decorin).[95,96] Finally, the mechanical strength of acellular ECMs was found comparable to native tissues, indicating that acellular ECMs can provide mechanical properties required to reconstruct the desired tissue.[97] Nonetheless, better understanding of the intrinsic biochemical and biomechanical properties of a particular decellularized matrix in regulating MSC fate decisions is still in need.

Pluripotent stem cell proliferation and differentiation on tsECMs

Similar to MSCs, seeding of PSCs on tsECMs induces the commitment into the lineages of ECM tissue sources (Table 3). For instance, decellularized cardiac matrices were reported to preserve heart tissue signaling network and induce hESC differentiation toward cardiac lineage.[98] Reconstructing myocardium and vascular structures was demonstrated using human iPSC-derived cardiovascular progenitor cells to repopulate the decellularized heart matrices.[28,99] The heart construct responded to β-adrenergic agonist isoproterenol and displayed electrophysiology and mechanical properties of heart tissue.[28] Decellularized matrices derived from lung or kidney were reported to promote the commitment of ESCs and iPSCs into epithelial tubules or renal cells.[100–103] The ECMs derived from the fibroblast feeders were also shown to support the self-renewal of hPSCs during long-term culture,[52,59,86,104] while the ECM of ARPE19 cells increased hPSC differentiation toward retinal pigment epithelial cells.[105] Proteomics analysis identified heparin sulfate proteoglycan as a core ECM component responsible for hPSC self-renewal.[86] The ECM composition and the controlled modulus of islet cell-derived matrices have shown to direct hESC differentiation into islet β cells.[106] These data indicate that tsECMs can at least partially recapitulate the signaling network of the tissue source to support stem cell self-renewal or direct lineage-specific differentiation.[29,107]

Signaling Mediated by scECMs

Different from tsECMs, scECMs contain unique signaling networks that regulate self-renewal and lineage specification during tissue development and recapitulate specific stem cell microenvironment.[12] Understanding these signaling networks should better control the *in vitro* culture environment and coax the *in vivo* development of the transplanted stem cells.

Endogenous signaling in MSC-derived extracellular matrices

MSCs secrete a large amount of ECMs forming an instructive microenvironment *in vivo* and *in vitro* for self-renewal and lineage commitment (Fig. 2). At an undifferentiated state, MSCs were found to secrete collagen type I, collagen type IV, vitronectin, and laminin.[18,108] During adipogenic differentiation, MSCs were shown to increase the expression levels of collagen type III, decorin, and nidogen.[109] Chondrogenic differentiation of MSCs was reported to increase the secretion of collagen type I, II, X, aggrecan, and cartilage oligomeric protein.[110] Differently, MSC osteogenic differentiation was shown to enhance the secretion of collagen IV, laminin, hydroxyapatite, calcium, and magnesium salt.[110, 111]

MSCs also secrete a large amount of growth factors, such as TGF-β1, FGF-2, VEGF, and PDGF,[112] which interact with ECMs and participate in the paracrine and autocrine signaling that regulate MSC proliferation and differentiation.[113] In addition, the culture time and donor age may play a primary role in the quality of MSC-derived ECMs.[114] Upon osteogenic differentiation, MSCs upregulate the secretion of stromal cell-derived factor (SDF)-4, connective tissue growth factor (CTGF), BMP-2, and FGF-18, as autocrine signaling to promote osteogenesis.[46,115–117] However, osteogenic and chondrogenic differentiations of MSCs were reported to reduce the secretion of proangiogenic factors, for example, PDGF, TGF-β, and FGF-2, and neurotrophic factors, for example, brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor.[118] Using microfluidics-based chambers, MSCs can secrete endogenous regulatory factors that elevate adipogenic genes CEBPA, CEBPB, PPARG, and LPL, while the exact factors were not reported.[119]

Besides growth factors, MSCs and their derivatives also differentially secrete antioxidant molecules such as superoxide dismutase protein SOD3, which may endogenously regulate cell survival and proliferation.[120] The MSC-derived ECMs also exhibit the antioxidant effects and were reported to decrease the intracellular levels of reactive oxygen species in reseeded MSCs, which may contribute to the increased cell proliferation and survival.[121,122] However, the intrinsic signaling in MSC-derived ECMs still needs to be further characterized.

Endogenous signaling in PSC-derived ECMs

Similar to MSCs, PSCs produce a large amount of endogenous ECM proteins, such as fibronectin, laminin, collagen type IV, vitronectin, and GAGs, which regulate PSC fate decision through cell adhesion and/or binding with paracrine and autocrine factors (e.g., Lefty and Activin A) (Fig. 3).[62,123–126] The paracrine and autocrine factors (e.g., Lefty and TGF-β) have been shown to deposit in endogenous ECMs[65,125,127–129] and regulate the survival and the repopulation ability of neural progenitors.[130,131] The characteristics of PSC-derived ECMs can be influenced by lineage specifications.[62,132,133] For example, the expression level of ECM proteins was found to be upregulated upon spontaneous differentiation.[19] Cerberus, a small antagonist of BMP, was detected in the secretome and ECMs of PSCs undergoing cardiac differentiation but not neural differentiation.[62,134] The lineage-specific cells derived from PSCs, such as NPCs, are known to secrete trophic factors such as BDNF and FGF-2 to stimulate neurogenesis.[135,136] The ECMs derived from PSC aggregates at early or late stages of differentiation exhibit different signaling capacities, suggesting the influence of developmental stage on ECM characteristics.[19,20,63,70,134] For instance, the upregulation of collagen type IV and laminin was found during endodermal specification.[63] The specific lineage differentiation of ESCs into ectodermal (using retinoic acid) or mesodermal (using BMP-4) cells can further enhance the expression of fibronectin, vitronectin, and collagen type IV.[19]

Concomitantly, PSCs also secrete a large amount of endogenous growth factors, which regulate PSC self-renewal and lineage commitment.[125] For instance, hESCs were

Table 3. Pluripotent Stem Cell Expansion and Differentiation on Decellularized Matrix Derived from Tissues or Cultured Somatic Cells

ECM source	*PSC source*	*Decellularization method*	*Properties of the acellular matrix*	*Effect on PSC proliferation and differentiation*	*Ref.*
Tissues					
Kidney and lung	hESCs	Chemical treatment: 1% SDS, Triton X-100	Preservation of dermcidin, defensin, TGF-β, EGF-7, and heparin-binding growth factor-2; collagen, keratin, vimentin, vitronectin	Epithelial tubule (DPEP1, HS6T1, CLCN7, FABP1 etc.); lung differentiation (BPI and MUC5B)	Nakayama *et al.*[100]
Kidney	mESCs	Chemical treatment: Triton X-100 and SDS, DNase, sodium chloride	Preservation of collagen type IV and laminin	Differentiation into endothelial cells and epithelial cells (renal cells, Pax-2, and Ksp-cadherin expression); lumen formation	Ross *et al.*[101]
Kidney	mESCs	Chemical treatment: SDS, Triton X-100, benzonase	Retained laminin, fibronectin, collagen type IV, and heparan sulfate proteoglycan; retained tubules, blood vessels, and glomeruli structures	mESCs differentiated into metanephric mesenchyme cells matured into kidney cells (cytokeratin and KSP expression) and formed tubule morphology	Sambi *et al.*[102]
Kidney	hiPSCs	Chemical treatment: SDS, DNAse	Persevered laminin and collagen I; retained heparin-binding growth factors (VEGF, FGF-2, BMP-2, HGF, PDGF-BB, TGF-β)	Increased endothelial differentiation (CD144 and CD31 coexpression) on dECM with VEGF	Ullah *et al.*[181]
Heart	hESCs	Chemical treatment: SDS	Contain collagen I to collagen VI, elastin, fibrinogen, fibronectin and laminin, fibrillin-1, fibulin-5, proteoglycans, and decorin heparan sulfate	Cardiac differentiation (Titin M8, desmoplakin expression)	DeQuach *et al.*[182]
Heart	hiPSC derived cardio-vascular progenitors	Chemical treatment: SDS followed by Triton X-100	Typical ECM proteins such as fibronectin, laminin, and collagen II remained; ECM had filament-like appearance	About 70% cardiomyocytes; normal electrophysiology and drug response, expression of sarcomeric α-actinin and cardiac troponin T, etc.	Lu *et al.*[28]
Heart	hiPSCs	Chemical treatment: SDS	Retention of collagen type IV and elastin	Improved cardiac maturation (α-sarcomeric actinin, troponin T, myosin heavy chain Nkx2.5)	Garreta *et al.*[99]
Heart	hiPSCs	Chemical treatment: SDS, Triton×100, EDTA	Preservation of fibrinogen, laminin, and collagen III; preservation of the ECM texture and orientation of the decellularized heart	Improved cardiac maturation (e.g., expression of cTnT and MYH6, atrial markers)	Wang *et al.*[183]
Ventricle heart or umbilical cords	hiPSC-derived cardiomyocytes	Chemical treatment: SDS (heart), CHAPS-EDTA (umbilical cord)	N.D.	Increased contractility on decellularized heart tissues; better cell alignment on decellularized umbilical cords	Park *et al.*[184]
Heart	hiPSC-derived cardio-myocytes	Physical/chemical treatment: hyper/hypo tonic NaCl, trypsin, Triton X-100, lyophilization and solubilization in HCl and pepsin	Retained collagen type I III and GAG, the mechanical properties of dECM was similar to adult heart	Increased contractile function (TNNT2, MYL2/MYL7 ratio), excitation–contraction coupling (CASQ2); better cellular alignment	Goldfracht *et al.*[185]

(*continued*)

Table 3. (Continued)

ECM source	*PSC source*	*Decellularization method*	*Properties of the acellular matrix*	*Effect on PSC proliferation and differentiation*	*Ref.*
Anterior tibial muscle (ATM) and ventricle tissues	mESCs (clone αPIG44)	Chemical treatment: SDS, Triton X-100, trypsin	Preserved collagen fiber network. Elastin was only preserved on ventricle tissue-derived ECMs	Retain cell proliferation; differentiation into functional cardiomyocytes (α-MHC, MLC2v, ANP, and field potential)	Hong *et al.*[186]
Liver	Porcine iPSCs	Chemical treatment: SDS	Preservation of collagen and GAGs; more than 40 different liver-derived growth factors were preserved after liver decellularization	Increased maturation of iPSC-hepatoprogenitor toward hepatocyte phenotype (AFP and ALB)	Park *et al.*[187]
Liver	miPSCs	Physical/chemical treatment: Pressurization at 1000 MPa, Dnase I, $MgCl_2$, EDTA	Preservation of collagen type III and of the mechanical properties of the native tissue	Increased hepatogenic differentiation (AFP, ALB, TAT, etc.)	Hirata and Yamaoka[67]
Liver	hiPSCs	Chemical treatment: SDS, Triton X-100, pepsin	Preservation of collagen and GAGs	Increased expression of hepatogenic makers: CYP3A4, CYP2B6, FOXA1/2, HHEX, HNF4, LHR1, PBDC1, CK18, AFP, HNF6, CEBDP, albumin	Jaramillo *et al.*[103]
Pancreas	miPSCs	Chemical treatment: Triton X-100, ammonium hydroxide	Preservation of collagen type I, collagen type IV, laminin, fibronectin, sGAGs	Improved maturation into pancreatic β cells (insulin expression)	Wan *et al.*[188]
Cultured cells					
Fibroblasts	hESCs	Chemical treatment: NH4OH treatment	Collagen I, collagen III, fibronectin, and HSPG	Support long-term self-renewal (Oct-4, NANOG, and SOX-2 expression)	Abraham *et al.*[86,104]
Fibroblasts	hESC	Chemical treatment: Triton X-100, NH_4OH, genipin crosslinking	Preservation of fibrinectin, laminin, collagen type I, and vitronectin	Retained proliferation and self-renewal on hard dECM, soft dECM promotes commitment and EMT	Kim *et al.*[59]
RIN5F cells (islet cell line)	hESCs	Chemical treatment: NH_4OH treatment	Collagen IV, fibronectin and laminin	Insulin secreting β cell differentiation	Narayanan *et al.*[106]
Mouse embryonic fibroblasts (MEF)	mESCs	Chemical treatment: ammonium hydroxide, Triton X-100	Retained fibronectin and collagen type I; the stiffness and amount of fibronectin and collagen type I on ECM were a function of the initial gelatin concentration used for MEF expansion	ECM from MEF reduced Oct-4 expression as a function of the ECM stiffness; Increased osteogenic differentiation in an ECM stiffness-dependent manner	Sthanam *et al.*[52]
Retinal pigment epithelium cells (ARPE19 cell line)	hiPScs	Chemical treatment: deoxycholate, DNAse	Comparable composition (fibronectin, vitronectin, collagen IV and V, laminin-α5) and microstructure than Buch's membrane	Similar expression of PAX-6, MITF, and OTX2 to culture on geltrex, but increased expression of RPE65	McLenachan *et al.*[105]

AFP, alpha-fetoprotein; ALB, albumin; ANP, atrial natriuretic peptide; BPI, bactericidal/permeability-increasing protein; CEBP, CCAAT enhancer-binding protein; CK, cytokeratin; CLCN7, chloride voltage-gated channel 7; cTnT, troponin T; CYP, cytochrome P450; DPEP1, dipeptidase 1; EMT, epithelial to mesenchymal transition; FABP1, fatty acid-binding protein 1; FOX, forkhead box protein; hESC, human embryonic stem cell; hiPSCs, human induced pluripotent stem cells; HNF, hepatocyte nuclear factor; HS6T1, heparan sulfate 6-O-sulfotransferase 1; HSPG, heparin sulfate proteoglycan; KSP, kinesin spindle protein; LHR1, liver receptor homolog 1; mESC, mouse embryonic stem cell; α-MHC, myosin light chain α; miPSC, mouse-induced pluripotent stem cell; MITF, microphthalmia-associated transcription factor; MLC2v, myosin regulatory light chain 2; MUC5B, mucin 5B; MYH, myosin heavy chain; MYL, myosin light chain; Nkx2.1, NK2 homeobox 1; Nkx2.5, NK2 homeobox 5; OTX2, orthodenticle homeobox 2; Pax-2, paired box gene 2; PAX-6, paired box protein 6; RPE65, retinoid isomerohydrolase; sGAG, sulfated glycosaminoglycan; TAT, tyrosine aminotransferase; TNNT2, troponin 2.

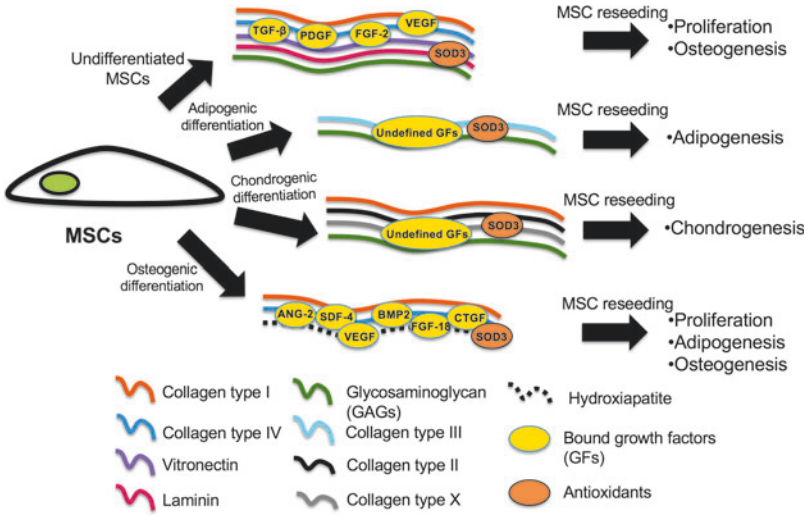

FIG. 2. The specific composition and biological functions of MSC-derived ECM. MSCs secrete the specific combination of ECM proteins and growth factors at undifferentiated and various differentiated stages. Decellularization of MSC-secreted ECM complexes can provide 3D scaffolds bearing unique biochemical and biological signaling in stem cell microenvironment. Color images are available online.

reported to secrete low levels (ng-pg/mL) of IGF-2, TGF-β1, FGF-2, HGF, nerve growth factor, and Wnt ligands to sustain the self-renewal.[137] Differently, mouse ESCs were reported to secrete leukemia growth factor (LIF), Lefty, Nodal, Wnt, and cyclophilin A, which can support self-renewal and OCT-4 expression.[134,138] The paracrine factor FGF-4 was also reported for early commitment of mouse ESCs.[139] The secreted growth factors have been shown to actively interact with ECMs in PSC culture. For example, the presence of regulators in TGF-β/Nodal and Wnt signaling pathways, Lefty A and B, Cerberus, and sFRP1/2, were observed in hPSC-conditioned matrix.[62,84] These studies demonstrate the presence of a unique spectrum of signaling molecules in PSC-derived ECMs.

Biological functions of scECMs

scECMs provide a stimulating microenvironment for reseeded cell expansion, differentiation, and cytokine secretion (Table 4). MSC-derived ECMs were reported to improve MSC proliferation compared with 2D plastic dishes, possibly through antioxidant molecules and bound growth factors, such as FGF-2, TGF-β, and VEGF.[12,18] Decellularized ECMs from MSCs were also reported to upregulate the expression of integrins α2 and β5 and activate ERK1/2 signaling in the reseeded MSCs.[121] In addition, ECMs derived from MSCs can increase osteogenic differentiation, potentially through the retained growth factors (e.g., BMP-2) and the combination of ECM proteins (e.g., collagen type I).[18,32,94,122] Indeed, proteomics analysis reveal that the composition of ECM proteins (e.g., tenascin C, vitronectin, fibronectin, etc.) and immobilized molecules (e.g., TGF-β, CTGF, CYR61) are responsible for the osteoinductive properties of MSC-ECMs.[107] The chondrogenic potential of MSCs can be enhanced on MSC-derived ECMs possibly due to the upregulation of TGF-β receptor II.[121] MSC-ECMs have also been found to stimulate cytokine secretion, including angiopoietin-1 (ANG-1), SDF-1, and IL-8 from the reseeded MSCs, and ANG-2, VEGF, and

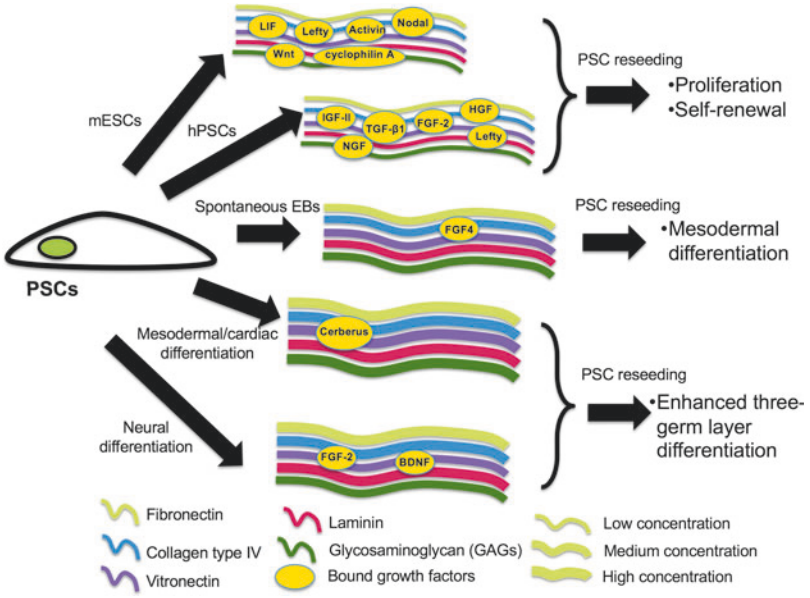

FIG. 3. The specific composition and biological functions of PSC-derived ECM. PSCs secrete the specific combination of ECM proteins and growth factors at undifferentiated and various differentiated stages. Decellularization of PSC-secreted ECM complexes can provide 3D scaffolds bearing signaling specificity along the embryonic tissue development. PSC, pluripotent stem cell. Color images are available online.

Table 4. Decellularized Extracellular Matrices Derived from Stem Cells and Their Biological Function

ECM source	*Reseeded cell source*	*Decellularization method and properties of the acellular matrix*	*Effect on stem cell proliferation*	*Effect on stem cell differentiation*	*Ref.*
MSCs					
BM-MSCs	BM-MSCs	Chemical treatment: Triton X-100, NH_4OH; expression of collagen I, collagen III, fibronectin, laminin	Fivefold increase in proliferation; fourfold less expression of reactive oxygen species	Increase hepatogenic differentiation (increase urea secretion)	He *et al.*[189]
Dental pulp MSCs	Dental pulp and periodontal ligament stem cells	Chemical treatment: Lysis followed by DNA digestion; retained BMP-2, TGF-β, VEGF, and PEDF, fibronectin	N.D.	Increased odontoblastic differentiation (DMP-1, DSP, and DPP expression)	Ravindran *et al.*[190]
BM-MSCs	BM-MSCs	Chemical treatment: Triton X-100, NH_4OH, DNAse; retained FGF-2, fibronectin, laminin, vitronection, collagen type I, and IV	Increased proliferation	Increased osteogenic differentiation (Alizarin Red S and von Kossa staining)	Kim and Ma[18]
BM-MSCs	BM-MSCs	Chemical treatment: Triton X-100, DNAse; preserved fibronectin biglycan, Decorin and collagen type I	N.D.	Increase osteogenic differentiation; in an integrin-dependent manner (increased RUNX2 and osterix expression)	Decaris *et al.*[94]
BM-MSCs	BM-MSC	Chemical treatment: Triton X-100 and NH4OH, DNAse; no characterization of the ECM	Increased MSC proliferation; upregulated SSEA-4 and integrin α2 and β5 expression	Enhanced chondrogenic differentiation (GAGs, phospho-TGF-β RII, SOX-9) and osteogenic differentiation (Alizarin Red D and ALP staining); decreased adipogenic differentiation (Oil Red O staining)	Pei *et al.*[121]
BM-MSCs	BM-MSCs; hematopoietic stem cells	Chemical treatment: Triton X-100, ammonium hydroxide; retained collagen, sGAGs, HGF, FGF, and VEGF	Increased MSC proliferation; Increased $CD34^+$ cell expansion, supported long-term engraftment	Increased osteogenic (von Kossa staining) and adipogenic differentiation (Oil Red O staining); Increased cytokine secretion (ANG-1, SDF-1, IL-8)	Prewitz *et al.*[12]
Osteogenically induced BM-MSCs	BM-MSCs	Chemical treatment: Triton X-100, NH_4OH, and DNAse; no characterization of the ECM	Reduced proliferation	Increased osteogenic differentiation (ALP, Alizarin Red S, IBSP, RPL13 expression) and proangionenic (VEGF) function	Hoch *et al.*[25]
BM-MSCs	BM-MSCs	Chemical treatment: Triton X-100 containing NH_4OH; increased roughness of the ECM in comparison to CELLstart™	Increased MSC proliferation under serum-free conditions; Increased colony formation	Increased osteogenic differentiation (ALP, BSP, and Runx2 expression)	Rakian *et al.*[191]

(*continued*)

TABLE 4. (CONTINUED)

ECM source	*Reseeded cell source*	*Decellularization method and properties of the acellular matrix*	*Effect on stem cell proliferation*	*Effect on stem cell differentiation*	*Ref.*
Immortalized cell lines: CMSC2, DMSC23 (hTERT-MSCs), 3T3 line	Placenta-derived MSCs	Chemical treatment: Triton X-100, NH_4OH; preservation of collagen type I, fibronectin and proteoglycans	ECMs derived from DMSC23 cells promote proliferation than the ECMs derived from CMSC2 and 3T3 cells	ECMs derived from DMSC23 promoted osteogenic differentiation compared with CMSC2 and 3T3 cells (Alizarin Red S and Osteoimage staining)	Kusuma *et al.*[192]
Adipogenically-induced A-MSCs	A-MSCs	Chemical/physical treatment: Triton X-100, NH_4OH, DNAse, RNAse freeze/thaw cycles, glutaraldehyde, glycine; increased laminin, and decreased fibronectin	Retained proliferation, increased number of CFU-Fs	Increased adipogenic differentiation (increased FABP4 and PPAR-γ expression, measured by RT-qPCR)	Zhang *et al.*[30]
AC and chondrogenically induced A-MSCs and BM-MSCs	A-MSCs and BM-MSCs	Chemical treatment: Triton X-100, NH_4OH, DNAse; persevered collagen type I and II, fibronectin, aggrecan and hyaluronic acid, and fiber organization	Chondrogenic-induced derived -dECM better promoted proliferation than AC induced derived ECM	Chondrogenic-derived dECM promoted chondrogenic differentiation (expression of collagen II and aggrecan); AC induced derived ECM promoted osteogenic differentiation	Perez-Castrillo *et al.*[31]
Proliferative, adipogenically and osteogenically induced A-MSCs	A-MSCs	Physical/chemical treatment: Triton X-100, NH_4OH, freeze/thaw, gluataraldehyde, glycine; preserved fibronectin, laminin, collagen type I, collagen type IV, reduced GAGs	Similar proliferation on dECM than on tissue culture plastics	Adipogenically induced dECM promoted adipogenic differentiation (PPAR-γ expression); osteogenically induced dECM promoted osteogenic differentiation (collagen I expression)	Guneta *et al.*[150]
BM-MSCs cultured on nanogrooves	BM-MSCs	Chemical treatment: Triton X-100, NH_4OH; preserved collagen I, II, and IV, fibronection and laminin. dECM fibers were aligned in the direction of nanogrooves	N.D.	Increased chondrogenesis on nanogrooves coated with dECM: reduced collagen type I and increased LAMB1 and aggrecan expression	Ozguldez *et al.*[155]
PSCs					
Embryoid bodies	3T3 fibroblasts	Mechanical treatment: Lyophilization or freeze/thaw cycling; no characterization of ECM composition	ECMs promoted fibroblast attachment, adhesion, and repopulation of matrices	N.D.	Ngangan and McDevitt[66]
ESC aggregates and embryoid bodies	mESCs	Chemical treatment: Triton X-100, DNAse; preservation of collagen IV, fibronectin, laminin, vitronectin, and GAG	No change in cell proliferation, except ECMs from retinoic acid-treated embryoid bodies	Increased three-germ layer differentiation (FOX-2 for endoderm; β-tubulin III for ectoderm; and α-actinin for mesoderm) on the 3D ECM scaffolds	Sart *et al.*[19]

(*continued*)

Table 4. (Continued)

ECM source	*Reseeded cell source*	*Decellularization method and properties of the acellular matrix*	*Effect on stem cell proliferation*	*Effect on stem cell differentiation*	*Ref.*
Embryoid bodies	mESCs	Chemical treatment: Triton X-100, SDS, SDC; retained collagen type I, IV, fibronectin, laminin, and sGAGs	Higher cellular attachment and proliferation	Supported three-germ layer differentiation (Brachyury, FGF-8, FGF-5, AFP, and Nestin expression) on the 3D ECM scaffolds	Goh *et al.*[33]
hESCs (H9 and CA1) and hiPSCs (BJ-1D)	hESCs	Chemical treatment: Cell recovery solution; retained frizzled-related proteins 1 and 2, Lefty A and B, connective tissue growth factor, Cerberus, and FGF-15	Support the maintenance of hPSC self-renewal	N.D.	Hughes *et al.*[62]
ESC aggregates, embryoid bodies, and ESC-derived neural progenitors	Neural progenitors derived from mESCs	Chemical treatment: Triton X-100, DNAse; no characterization of the ECM composition	ECMs derived from PSC-NPCs reduced proliferation of reseeded cells	ECMs derived from NPCs promoted neuronal differentiation; Crosslinking reduced neuronal differentiation (β-tubulin III expression) on the 3D ECM scaffolds	Sart *et al.*[20]
PSC aggregates and embryoid bodies and PSC derived neural progenitors	mESCs hiPSCs	Chemical treatment: Triton X-100, DNAse; ECMs derived from NPCs express more collagen IV alpha 2, laminin B1, glypican, activity-dependent neuroprotective protein, neuron-derived neurotrophic factor	ECM derived from PSC-neural progenitor reduced cell proliferation for both reseeded mESCs and hiPSCs	NPC group preferably induces neural differentiation for both reseeded mESCs and hiPSCs (Nestin and β-tubulin III expression) on the 3D ECM scaffolds	Yan *et al.*[84]
hESC-derived fibroblasts	hESCs	Chemical treatment: SDC, protease inhibitor; retained collagen type I and VII, fibronectin, laminin	Delayed senescence of keratinocytes derived from hESCs	Keratinocytes differentiation (e.g., K14, K10 expression)	Movahednia *et al.*[140]
hESC-derived neural progenitors	DFSCs	Chemical treatment: NH_4OH, deionized water, DNAse; retained GAGs, collagen I, fibronectin, and collagen type IV	N.D.	Enhanced neural differentiation on ECM derived from hESC-NPCs (PAX-6, NSE, Musashi-1 and β-tubulin III)	Heng *et al.*[141]

AC, ascorbic acid; A-MSC, adipose tissue-derived mesenchymal stem cells; ANG-1, angiopoietin 1; BM-MSC, bone marrow mesenchymal stem cells; DFSCs, dental follicle stem cells; DPP, dentine phosphophoryn; DSP, dentin sialophosphoprotein; IBSP, integrin-binding sialoprotein; IL-8, interleukin 8; K14, keratin 14; LAMB1, laminin subunit beta 1; NPC, neural progenitor cell; PEDF, pigment epithelium-derived factor; phospho-TGF-βRII, phosphorylated form of transforming growth factor beta receptor II; RPL13, ribosomal protein L13; SDF-1, stromal cell-derived factor 1.

HGF from reseeded hematopoietic stem cells, providing a mimetic bone marrow ECM environment that supports their long-term engraftment.[12]

Similarly, acellular matrices from different PSC organizations retain endogenous ECM proteins and exogenous morphogens such as retinoic acid, which can accelerate three-germ layer commitment through the regulation of retinoic acid receptor signaling (Table 4).[19,33] In addition, decellularized ECMs derived from undifferentiated ESCs support the reseeded ESC self-renewal in the absence of exogenous LIF.[19] This indicates a possible retention of autocrine regulatory molecules such as LIF on decellularized matrices. For hPSCs, endogenous ECMs deposited on Matrigel were shown to support the propagation of hPSCs due to the retention of the paracrine and autocrine factors such as Gremlin and Cerberus (antagonists of BMP signaling).[62] For directed differentiation, the ECMs of PSC-derived fibroblasts were reported to promote keratinocyte differentiation by modulating TGF-β1 signaling.[140] Of interest, it was recently found that the cues provided by ECMs of neural differentiated PSCs direct the differentiation of MSCs.[141] Altogether, these data indicate that scECMs retain the specific cues along tissue development and are able to uniquely regulate cellular differentiation, providing the dynamic reciprocal interactions among stem cells, ECM, and growth factors.

Engineering scECMs

Modulation of biochemical and biomechanical properties of ECMs

The functionality of acellular matrices can be improved by the addition of exogenous regulatory molecules that can bind to ECM proteins. For instance, the decellularized matrices could be functionalized by exogenous growth factors (e.g., FGF-2) bound to the heparin-binding domains of GAGs.[142] The newly retained growth factors demonstrate prolonged retention and controlled delivery to the reseeded stem cells.[142] Similarly, the retention of exogenous retinoic acid in ESC-derived ECMs promotes the commitment of reseeded ESC to the three germ layers.[19] Moreover, acellular ECMs can be functionalized with exogenous biomaterials such as collagen or hyaluronan, which were shown to promote PSC differentiation toward cardiac lineage.[143,144] The combination of inorganic materials and cell-derived ECMs results in the formation of novel hybrid scaffolds, providing synergic biochemical and biophysical cues regulating stem cell differentiation.[145]

The mechanical properties of ECM scaffolds can be modified by crosslinking. The crosslinking of decellularized matrices not only promotes ECM stability but also increases the elastic modulus, that is, stiffness.[146] The stiffness of ECMs modulated by crosslinking was reported to promote hypertrophic differentiation and matrix calcification of chondrogenically induced MSCs and enhance osteogenic differentiation.[147,148] In addition, crosslinking of endogenous ECM through glycation regulates the mechanical properties of ECMs without altering the osteogenic differentiation potential of the reseeded cells.[149] Among various crosslinking methods, crosslinking of acellular matrices by genipin was shown to have similar microstructure and mechanical properties but lower cytotoxicity compared with crosslinking by glutaraldehyde.[146] Our previous study shows that the stiffness of endogenous PSC-derived ECMs, modulated by genipin crosslinking, regulates neural specification of ESC-derived neural progenitors.[20] Similarly, it has been shown that the crosslinking degree of the scaffolds regulates early commitment of PSCs in a stiffness-dependent manner.[58]

Modulation of culture conditions before decellularization

Stem cell culture conditions regulate the secretory profiles of ECMs and growth factors. First, the directed differentiation of stem cells regulates the properties of ECMs that can induce commitment of reseeded undifferentiated cells toward the phenotype of cells that have been used to generate scECMs. For instance, the scECMs of adipogenically, osteogenically, or chondrogenically differentiated MSCs promote the differentiation of undifferentiated MSCs toward adipocytes, osteoblasts, or chondrocytes.[25,30,31,150] While the composition of differentiated scECMs needs characterizations, the results indicate that the scECMs contain specific structural and regulatory proteins that promote directed commitment of the reseeded cells. Similar observations have been made with the ECMs of PSCs that commit to neural differentiation, which can promote neuronal differentiation of the reseeded cells.[20,22,84,141]

While MSCs are usually grown and differentiated as monolayers, the MSC aggregates enhance the secretory profiles (e.g., VEGF, FGF-2, HGF, etc.) as well as the differentiation potential along adipogenic, chondrogenic, and osteogenic lineages.[151,152] Similarly, undifferentiated hESC aggregates showed the enhanced expression of E-cadherin, Tra-1-60, and Oct-4 compared with monolayers, indicating potential regulation of autocrine signaling by cellular organization.[153] Moreover, PSC and MSC aggregates were shown to regulate lineage commitment through differential accumulation of endogenous factors.[154] Indeed, confined microwell cultures containing accumulated factors were observed to induce endodermal and ectodermal specification, while suspension cultures with limited local concentrations of endogenous morphogens were observed to dominantly induce mesodermal specification.[154] Similarly, the nanogrooved surfaces regulate structural organization and the compositions of ECM fibers of MSCs, leading to chondrogenic differentiation of the reseeded cells.[155]

Dynamic cultures of stem cells in bioreactors or within biomaterials can modulate their secretome profiles as well. Indeed, undifferentiated MSCs display the enhanced secretion of VEGF and FGF-2 as well as ECM remodeling under flow shear stress.[156] Similarly, under exposure to mechanical stresses, hESCs secrete high levels of TGF-β1, Activin A, and Nodal, which can inhibit spontaneous differentiation.[157] In addition, oxygen tension is able to regulate the growth factor secretion from MSCs such as VEGF, PDGF, or FGF-2, and the secretion of collagen type I and fibronectin.[18,158] PSCs were shown to increase the secretion of VEGF under hypoxia (1% O_2).[159] Hence, modulation of stem cell culture conditions may increase the bioactivity of scECMs by regulating secretory function.[160]

Conclusions and Perspectives

scECMs have unique properties and have demonstrated significant potential in regenerative medicine. The wide-spread applications of scECM, however, depend on addressing several important questions underlying their functional properties. (i) First, in-depth characterization of the biochemical and biomechanical properties of scECM remains a challenge because of large variations in scECM derivation methods. Advanced proteomic tools will provide important insights in scECM properties and in understanding scECM–cell interactions. (ii) Additionally, scECMs directly obtained from stem cell cultures may have low mechanical strength and poor structural and functional stability, which significantly limit their applications. Methods that can functionalize scECMs while preserving their innate properties should significantly improve their properties. (iii) In most studies, scECMs were used as 2D substrates, whereas recent works have demonstrated that scECMs can also serve as 3D scaffolds (Table 4). Thus, there is a need to better characterize the bioactive function of scECMs depending on their configurations in cultures (2D vs. 3D). (iv) To date, scECM derivation has been primarily carried out in the laboratory with low efficiency and reproducibility. Massive production of scECM has been very challenging. Research on scalable and robust biomanufacturing methods should significantly improve the regeneration medicine and therapeutic use of scECMs. The advances in these areas play important roles in fully understanding ECM microenvironment of stem cells and in establishing technologies to obtain *ad equate* biomimetic ECM–stem cell constructs for clinical applications.

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Address correspondence to:
Yan Li, PhD
Department of Chemical and Biomedical Engineering
FAMU-FSU College of Engineering
Florida State University
2525 Pottsdamer Street
Tallahassee, FL 32310

E-mail: yli4@fsu.edu

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