

Expert Opinion

1. Introduction
2. The *in vivo* role of ECM
3. Decellularized matrices derived from tissues and organs
4. Cell-formed decellularized matrices
5. Cell-formed decellularized matrices for the regulation of stem cell functions
6. Conclusions
7. Expert opinion

informa
healthcare

Decellularized matrices for tissue engineering

Takashi Hoshiba, Hongxu Lu, Naoki Kawazoe & Guoping Chen[†]

National Institute for Materials Science, Biomaterials Center, Tsukuba, Ibaraki, Japan

Importance of the field: Biomimetic scaffolds and substrates of extracellular matrices (ECMs) play an important role in the regulation of cell function and in the guidance of new tissue regeneration, as an ECM has the intrinsic cues necessary to communicate with and dictate to cells.

Areas covered in this review: This paper reviews the latest developments in ECM scaffolds and substrates obtained from decellularized tissues, organs or cultured cells and their application in tissue engineering. The ECM composition, structure, interaction with surrounding cells, preparation method and usage in the regeneration of various tissues and organs are summarised.

What the reader will gain: The advantages and challenges of decellularized matrices are highlighted.

Take home message: Similarity in the composition, microstructure and biomechanical properties of the decellularized scaffolds and substrates to those of the native tissues and organs maximizes the promotion effect in the regeneration of both structural and functional tissues and organs. Simple tissues as well as complicated organs have been decellularized and decellularization methods have been optimized to completely remove the cellular components while keeping the ECM intact.

Keywords: decellularization, extracellular matrix, stem cell, tissue engineering

Expert Opin. Biol. Ther. (2010) 10(12):1717-1728

1. Introduction

By combining cells, biomaterial scaffolds and biologically active molecules, tissue engineering has been proven to be a promising therapeutic approach in treating disease and injury in light of a variety of problems confronting currently available treatment methods [1,2]. Three-dimensional scaffolds play an important role in tissue engineering to control cell functions and to promote the formation of new tissues and organs [3]. The scaffolds provide initial support to the seeded cells, localise the cells in the appropriate spaces, provide physical and biological cues for adhesion, migration, proliferation and differentiation, and assemble the propagated cells and secreted matrices into functional tissues and organs. The scaffolds will be replaced by the cells' own matrices during the regeneration. Therefore, the scaffolds should be biocompatible, biodegradable and provide appropriate signals for the seeded cells to orchestrate the process. Meanwhile, two-dimensional cell culture substrates used for the *in vitro* subculture of somatic and stem cells are also very important for tissue engineering. The substrate should have the capacity to expand the cells to the maximum amount without changing the phenotypes and characteristics of the expanded cells. These special requirements drive the development of scaffolds and substrates that have the same functions as *in vivo* extracellular matrix (ECM).

ECM and ECM-mimicking scaffolds and substrates are ideal candidates for tissue engineering because in our body cells lay on- or are surrounded by ECM [4]. The ECM functions as not only a supporting material but also as the regulator of cellular functions such as cell survival, proliferation, morphogenesis and differentiation [4,5]. Moreover, the ECM can modulate signal transduction activated by various bioactive

Article highlights.

- ECM and ECM-mimicking scaffolds and substrates are ideal candidates for tissue engineering.
- To design scaffolds and substrates for tissue engineering and cell culture applications, the tissue specificity of ECMs should be considered for the induction of proper cellular functions.
- To obtain scaffolds mimicking native ECM, cellular components are selectively removed by a decellularization treatment.
- The ECM scaffold of decellularized small intestinal submucosa (SIS) is one of the well-studied decellularized ECM scaffolds.
- Decellularized amniotic membrane is another well-studied decellularized ECM scaffold because of its easy availability.
- A number of tissue-specific decellularized matrices have been developed.
- The biomechanical properties of decellularized ECM scaffolds are also very important factors in tissue engineering.
- The microstructure of the ECM also plays an important role in tissue engineering.
- Whole organs have been decellularized to keep the similarity of composition, biomechanical properties, microstructure and shape of the native organs.
- Cell-formed matrices can be used as a new cell culture substrate after a decellularization treatment.
- Cell-formed decellularized matrices have been extensively studied for the regulation of stem cell functions.
- The decellularized matrices will be useful matrices for not only tissue engineering and biological therapy but also for basic biological research.

This box summarizes key points contained in the article.

molecules such as growth factors and cytokines [6,7]. The ECM molecules and networks change their compositions, structures and biomechanical properties according to each specific tissue and organ. Their compositions and properties also dynamically change and remodel during development. Ideally, the scaffolds and substrates used for tissue engineering and cell culture can provide the same or similar microenvironment to the seeded cells as that of an ECM existing *in vivo*. However, native ECMs are composed of many kinds of proteins and have very intricate structures. Although many researchers have been trying to identify the proteins of ECMs *in vivo* [8], there remain a number of unidentified proteins. Therefore, it is difficult to reconstruct a scaffold or substratum that has the same composition as that of an *in vivo* ECM by conventional physicochemical methods. In addition to the difficulty in mimicking the ECM composition, it is also difficult to mimic the complex microstructure and architecture of the *in vivo* ECM. Due to these difficulties, researchers have tried to use the ECM in the tissues and organs or ECM proteins produced by *in vitro* cultured cells after decellularization treatment (Figure 1).

Various decellularized matrices have been developed from different tissues and organs as well as cultured cells. Decellularized matrices contain tissue- or cell-specific ECM proteins and remain a tissue- or cell-specific microstructure. The decellularized matrices have been widely used for the engineering of functional tissues and organs such as cartilage, skin, bone, bladder, blood vessels, heart, liver, lung and etc. In this review, we summarise the trials to develop decellularized matrices after a brief introduction of the roles played by the ECM.

2. The *in vivo* role of ECM

The ECM is a complicated complex of many kinds of polysaccharides and proteins, such as collagens, proteoglycans and glycoproteins. Cells adhere to the ECM via receptors (e.g., integrins) to maintain tissue architecture *in vivo*. In addition to the maintenance of tissue architecture, ECMs can regulate cellular functions in several ways [5]. The association between ECM proteins and receptors like integrin can directly activate intracellular signalling pathways. For example, it has been reported that the signal transduction activated by integrins is required for the escape of detachment-induced apoptosis [9]. Integrin activates survival signal molecules such as Akt and extracellular-signal-regulated kinase (ERK) for cells to survive and proliferate [10,11]. Also, integrin can activate Rho kinase to assemble actin filaments [12]. The signal transduction activated by integrins differs according to the type of ECM molecules [11,12]. In addition to direct activation of signalling pathways, the ECM can also regulate cellular functions through indirect pathways. Two types of indirect mechanisms have been indicated. One mechanism to regulate cellular functions is the modulation of cytokine activity. In the liver, hepatocyte growth factor (HGF) is stored in the ECM in an inactive form. When the liver is damaged, the ECM is degraded by matrix metalloproteinase (MMP) to release HGF to interact with hepatocytes for their proliferation [13,14]. In addition to the storage of cytokines in the ECM, the ECM can regulate cytokine activity. Decorin can bind to bone morphogenic proteins (BMPs) to increase their activity [15]. In contrast, biglycan can bind to chordin, an antagonist of BMPs, to suppress BMP activity [16]. In the neural stem cell (NSC) niche, FGF-2 binds to N-sulphated heparan sulphate proteoglycans (HSPGs) to maintain the NSC phenotype [17]. Moreover, intracellular signalling activated by integrins can modulate signalling pathways activated by cytokines [6,7]. The other mechanism to regulate cellular function by the ECM is transduction of mechanical signals. When cells are cultured on substrate having different elasticity, the cells show different responses according to the elasticity of the substrate [18,19]. When mesenchymal stem cells (MSCs) are cultured on substrates mimicking the elasticity of nerve, muscle and bone, MSCs differentiate into neurons, muscle cells and osteoblasts, respectively [18].

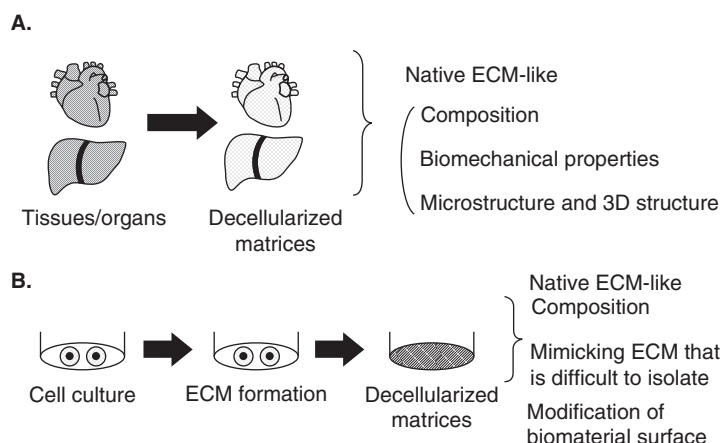


Figure 1. Decellularized matrices from tissues/organs and cultured cells, and their advantages. **A.** Decellularized matrices from tissues/organs. They have native-like ECM composition, microstructure and biomechanical properties. Also, they keep the shapes of the original tissues and organs. **B.** Decellularized matrices from cultured cells. They can mimic the composition of native ECM which is difficult to isolate from the tissues (e.g., stem cell niche). Biomaterial surface can also be modified with decellularized matrices from cultured cells.

The properties (e.g., composition, microstructure and elasticity) of ECMs vary depending on the type of tissue. The composition of the ECM is specialized in different tissues to exert tissue-specific cellular functions [8,20]. Moreover, the composition of ECM varies depending on different developmental stages and pathological conditions, like fibrosis, even within the same tissue [21-23]. In addition to the composition of the ECM, the microstructure varies depending on the type of tissue. For example, the microstructures of ECMs are different between epithelial tissue and stromal tissue [5]. The elasticity of the ECM is also different among the tissues [18]. To design scaffolds and substrates for tissue engineering and cell culture applications, the tissue specificity of ECMs should be considered for the induction of proper cellular functions.

3. Decellularized matrices derived from tissues and organs

3.1 Decellularized matrices and decellularization methods

ECMs and ECM-mimicking scaffolds have been shown to facilitate the constructive remodelling of many different tissues in both preclinical animal studies and in human clinical applications. To obtain scaffolds mimicking native ECM, cells in the tissue are specifically removed by a decellularization treatment and the intricate mixture of structural and functional proteins and glycosaminoglycan remains. Many methods have been developed for decellularization [24-29]. They include physical, chemical and enzymatic treatments. Physical treatments use agitation, sonication, mechanical massage, pressure or freezing and thawing to disrupt the cell membrane and release cell contents. Chemical treatments use alkalis, acids, detergents, organic solvents, chelating

agents, hypotonic solutions or hypertonic solutions to disrupt cell membranes and the bonds responsible for intercellular and extracellular connections. Enzymatic treatments use protease or nuclease to cleave the peptide or nucleotide bonds. All of these methods have their respective advantages and limitations. They can efficiently remove cellular components such as nuclei, actin and the cell membrane while they also have some adverse effects on the composition, biological activity, and biomechanical property of the remaining ECM. Generally, these methods are properly combined to maximize the decellularization effect while minimizing any adverse effect on the composition, biological activity, integrity and biomechanical properties of the remaining ECM. Appropriate methods should be selected and refined for the specific tissues and organs.

Decellularized ECM scaffolds from a variety of tissues, including heart [30] and heart valves [31-35], blood vessels [29,36,37], small intestinal submucosa (SIS) [36,38,39], lung [40-42], trachea [43], skin [44,45], nerve [46,47], cornea [48-50], oesophagus [51,52], liver [53-55], kidney [56], bladder [26,57], cartilage [58], ligament [28], adipose tissue [59-61] and amniotic membrane [62] have been prepared and tried for use in tissue engineering in many forms (Figure 2). Some of them have been commercialized for therapeutic applications. Among the commercially available ECM scaffolds, SIS- and skin-derived decellularized scaffolds are the most representative ones [39,44]. Some of the representative ECM scaffolds for each tissue are listed in Table 1. Applications for a few of the typical ECM scaffolds used in tissue engineering are discussed in detail below.

3.2 Decellularized matrices derived from small intestinal submucosa

The ECM scaffold of decellularized small intestinal submucosa (SIS) is one of the well-studied decellularized ECM

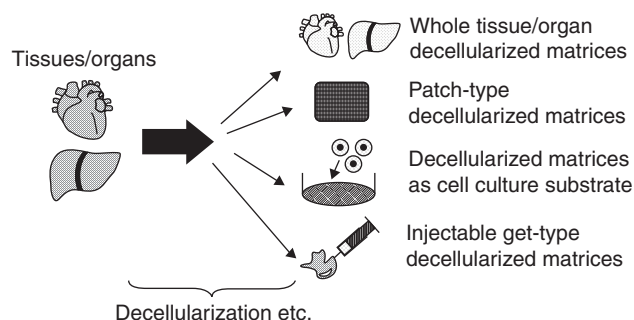


Figure 2. Different types of decellularized matrices derived from tissues and organs.

scaffolds [36,38,39]. The composition, microstructure and biomechanical properties of SIS-ECM scaffolds depend on the decellularization method. Collagens occupy almost more than 90% of the dry weight of decellularized SIS with type I collagen being the major type found. The SIS-ECM scaffolds contain a number of components: collagen, including type I, III, IV, V and VI; glycosaminoglycans (GAGs), including heparin, heparin sulphate, chondroitin sulphate and hyaluronic acid; adhesion molecules, such as fibronectin and laminin; proteoglycans including decorin; glycoproteins, such as biglycan and entactin; and various growth factors, including transforming growth factor- β (TGF- β), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). The SIS-ECM scaffolds have been used to reconstruct a variety of tissues including the integument, body wall, urinary bladder, rotator cuff, intestine, urethra, ureter, diaphragm [38] and blood vessels [34].

3.3 Decellularized matrices derived from amniotic membrane

Decellularized amniotic membrane is another one of the well-studied decellularized ECM scaffolds because of its easy availability [63]. Amniotic membrane is the innermost layer of the placental membranes. Amniotic membrane has both a basement membrane ECM side and a stromal ECM side. In the layer to which epithelial cells adhere, type I, type III and type IV collagens; laminins; and fibronectin are assembled. Human amniotic membrane has been widely used in many surgical procedures, such as skin transplantation, vaginal reconstruction and eye surgery [44,62]. Following clinical use, several beneficial effects, such as anti-inflammatory and antimicrobial effects, have been reported [64,65]. Various attempts have been made to modify human amniotic membrane, including chemical and physical cross-linking to enhance its physical properties and to remove cellular components [63].

3.4 Decellularized matrices for specific applications

The SIS-ECM and amniotic membrane have been used in clinics for various tissues, and not limited to one specific tissue. Because the composition and character of the ECM

of each tissue are different, tissue-specific ECM scaffolds are required for some tissues and organs. In other words, cellular functions may differ depending on the decellularized matrices derived from different tissues or organs. Badyal and colleagues reported that sinusoidal endothelial cells can maintain their differentiated functions on liver-derived decellularized matrices but not on SIS-derived and bladder-derived decellularized matrices, demonstrating the effect on cellular functions of decellularized matrices derived from different tissues [20]. Therefore, the source of the tissues or organs used in the preparation of decellularized matrices should be carefully chosen based on the intended usage and application. A number of tissue-specific decellularized matrices have been developed.

Primary hepatocytes lose their liver-specific functions, such as albumin secretion and drug metabolising activities, when they are cultured *in vitro*. The loss of their specific functions is one of the major problems for liver tissue engineering. To maintain the liver-specific functions of primary hepatocytes, Reid *et al.* developed decellularized matrices from liver tissue after homogenization. This decellularized matrix contains mostly liver collagens and an unknown quantity of noncollagenous components and proteoglycans. On this decellularized matrix, primary hepatocytes can attach and maintain their differentiated functions, like albumin secretion, for up to three months [53,54]. The authors claimed that this matrix might be one of the appropriate substrates for hepatocyte adhesion *in vitro*.

Decellularized fat tissue has been investigated in combination with mesenchymal stem cells for adipogenic applications [59,60]. Fat matrix contains collagens type I – VI, as well as other ECM components, such as laminin and fibronectin [66]. Culture of human adipose-derived stem cells in a decellularized fat ECM scaffold indicated that the decellularized adipose tissue provided an inductive microenvironment for adipogenesis, supporting the expression of the master regulators peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT/enhancer binding protein (C/EBP α), without the need for exogenous differentiation factors.

3.5 Decellularized matrices with appropriate biomechanical properties

The biomechanical properties of decellularized ECM scaffolds are also very important factors in tissue engineering. Acellular xenogeneic or allogeneic matrices have been used for tissue-engineered heart valves because the biomechanical properties of a valve can potentially be preserved with an optimal decellularization method that removes the cells while keeping the matrix intact. Steinhoff *et al.* decellularized sheep pulmonary valve conduits and seeded the scaffolds with autologous myofibroblasts and endothelial cells for reconstruction of viable pulmonary valve leaflets [31]. Six tissue-engineered valves and four unseeded control valves were implanted in sheep for 3 months. The tissue-engineered valves showed complete histological restitution of valve tissue and confluent endothelial surface coverage in all cases while the unseeded allogeneic

Table 1. Partial list of tissue/organ-derived decellularized matrices for tissue engineering.

Target tissues	Sources of matrices	Use	Ref.
Heart	Rat whole heart	Heart transplantation	[30]
Heart valves	Porcine aortic valves	Valvular heart disease	[31]
	Pig aortic valves	Bioprosthetic valves	[33]
	Procine myocardial tissue	Injectable gel for cardiac tissue engineering	[35]
Blood vessels	Pig carotid arteries	Vascular graft	[29]
	Dog small intestinal submucosa	Vascular graft	[36]
	Porcine aortic blood vessel	Vascular graft	[37]
Lung	Rat whole lung	Lung transplantation	[40]
	Rat whole lung	Lung transplantation	[41]
	Mouse whole lung	Lung bioreactor system	[42]
Trachea	Yorkshire boar trachea	Airway replacement	[43]
Skin	Human dermal tissue	Abdominal wall, breast, ear, nose and throat/head and neck reconstruction, grafting	[44]
Nerve	Peripheral nerve	Peripheral nerve graft	[46]
	Rat peripheral nerve	Peripheral nerve graft	[47]
Cornea	Human cornea stroma	Cornea transplantation	[48]
	Porcine cornea	Cornea transplantation	[49,50]
Esophagus	Porcine aorta	Patch for esophageal injury	[51]
	Rat esophagus	Artificial esophagus	[52]
Liver	Rat homogenized liver	Hepatocyte culture	[53,54]
	Ischemic rat liver	Liver transplantation	[55]
Kidney	Rhesus monkey kidney	Scaffold for kidney tissue engineering	[56]
Bladder	Dog bladder submucosa	Bladder augmentation	[26]
	Porcine bladder	Scaffold for bladder tissue engineering	[57]
Cartilage	Porcine menisci	Scaffold for meniscal repair	[58]
Ligament	Porcine anterior cruciate ligament	Bone-anterior cruciate ligament-bone graft	[28]
Adipose tissue	Rat adipose tissue	Adipogenesis of mesenchymal stem cell	[59]
	Human adipose tissue	Adipose tissue regeneration	[60]
	Rat skeletal muscle	Adipose tissue regeneration	[61]
Vagina	Human amniotic membrane	Vaginal reconstruction	[62]
Decellularized small intestinal submucosa has been used for the reconstruction of integument, body wall, urinary bladder, rotator cuff, intestine, urethra, ureter and diaphragm			[38]

acellular control valves showed partial degeneration (two of four valves) and no interstitial valve tissue reconstitution. Bertipaglia *et al.* cultured aortic valve interstitial cells in decellularized valve leaflets [32]. After culturing for two weeks, the cells penetrated the decellularized scaffolds, and a heterogeneous mesenchymal cell population of valve interstitial cells (fibroblasts, myofibroblasts, smooth muscle cells and endothelial cells) was present. The decellularized valve leaflets showed the cellular potential to reconstruct a viable aortic valve. Tedder *et al.* reported the usage of decellularized porcine pericardium and porcine pulmonary arteries for heart valve tissue engineering [33]. The two decellularized scaffolds were layered together to form a valve-shaped construct that was seeded with human bone-marrow-derived stem cells and cultured in a bioreactor. The cells in the ECM scaffolds showed similar morphology and properties to native human valve interstitial cells, indicating the feasibility of the approach.

3.6 Decellularized matrices with appropriate microstructures

In addition to the similarity of composition and biomechanical properties, the microstructure of the ECM also plays an important role in tissue engineering. Decellularized matrices

of tissues and organs can mimic the microstructure of native ECM. Decellularized cornea stroma was used for a culture of human corneal endothelial cells for cornea tissue engineering. After 14 days culturing in growth medium, the seeded human corneal endothelial cells retained the expression of the functional markers of corneal endothelial cells, and the engineered cornea showed similar biomechanical properties to the normal cornea [48]. For corneal tissue engineering with decellularized matrices, transparency is an important factor. To control the transparency of decellularized matrices, high hydrostatic pressure has been used to remove the cellular component of the cornea [49,50]. These decellularized matrices became clear after transplantation into the cornea [50]. It was suggested that the structure of the aligned collagen fibrils after decellularization was involved in producing the transparency after transplantation.

3.7 Decellularized matrices of whole organs

Most recently, whole organs have been decellularized to keep the similarity of composition, biomechanical properties, microstructure and shape of the native organs. The decellularized organ-derived ECM scaffolds have been used to reconstruct whole organs by re-seeding cells in the matrices [30,40-42,55].

Ott *et al.* first reported decellularized matrices derived from whole rat heart [30]. To keep the structure of the native ECM in the heart, the decellularization is performed with a perfusion technique. After the seeding of cells in the whole heart decellularized matrices, macroscopic contractions were observed within 4 days. By 8 days, the constructs could generate pump functions. Similar to whole-heart decellularized matrices, decellularized matrices derived from whole rat liver were developed by perfusion decellularization [55]. After the seeding of hepatocytes in the whole-liver decellularized matrices, hepatocytes showed liver-specific functions such as albumin secretion, urea synthesis and the expression of drug metabolising enzymes. Additionally, these constructs are transplantable. In 2010, three groups reported simultaneously the decellularization of a whole lung and the use of the decellularized lung for lung tissue engineering [40-42]. They used a detergent perfusion method to decellularize rat lungs and yield acellular lung matrices with intact vasculature, airway and alveoli structures. Lung cells and endothelial cells were seeded into the acellular lung matrices to repopulate airways and blood vessels, respectively. When the cell-seeded lung matrices were cultured in a bioreactor simulating the physiological environment of living lung, they generated gas exchange similar to that of isolated native lungs. After being transplanted orthotopically, the engineered lungs enabled ventilation, perfusion and gas exchange for several hours. These results may suggest that the use of an acellular organ matrix is a prospective approach for organ regeneration.

3.8 Decellularized ECM-incorporated gel scaffolds

In addition to the direct usage of decellularized ECM scaffolds for clinical applications, they can be incorporated in soluble-form materials that can be induced to self-assemble into a gel after the injection into the body. Such scaffolds have been recently focused on because they can theoretically fit to any three-dimensional spaces and can be delivered via minimally invasive surgical techniques. A few types of injectable gels composed of decellularized matrices of tissues and organs have been developed for tissue engineering applications. Freytes *et al.* reported injectable gel derived from decellularized matrices of porcine urinary bladder matrix [67]. This matrix was induced to self-assemble into a gel under physiological conditions. And the gel can support the adhesion and growth of rat aortic smooth muscle cells. Singelyn and colleagues developed injectable gel derived from decellularized matrices of myocardial tissues for cardiac tissue engineering [35]. The matrix could form a gel after *in vivo* injection. After the injection, endothelial cells and smooth muscle cells migrated towards the gel with a significant increase in arteriole formation.

4. Cell-formed decellularized matrices

Although decellularized matrices derived from tissues and organs have many advantages in their composition,

microstructure and biomechanical properties, the availability of tissues and organs for decellularization is problematic. Also, tissue or organ decellularization is not suitable for the preparation of decellularized matrices mimicking an ECM that is specific to a region of tissue, such as stem cell niches. To solve these problems, cell-formed matrices have been developed. When cells are cultured *in vitro*, ECM proteins are secreted from the cells and are then deposited beneath the cells [68,69]. Using this phenomenon, an ECM can be formed *in vitro*. After the formation of an ECM by cultured cells, cell-formed matrices can be used as a new cell culture substrate after a decellularization treatment. Cell-formed decellularized matrices have been reported since the 1970s [70,71]. Chen *et al.* reported a cell-free substratum containing matrices prepared from culture of chick embryo fibroblasts by decellularization treatment with non-ionic detergent, NP-40, at pH 9.6 [70]. Hedman *et al.* also isolated the pericellular matrices of human fibroblast by decellularization [71]. These studies indicate that the decellularized matrices can be used as a new cell culture substrate. Now, there are many types of cell-formed matrices which are prepared under various culture conditions (Figure 3). Some of the representative decellularized matrices derived from cell cultures are listed in Table 2.

Based on these studies, cell-formed decellularized matrices have been used and evaluated for their functions as new cell culture substrates. Mochitate and colleagues developed a new basement membrane model formed by alveolar epithelial cells [72-75]. They cultured alveolar epithelial cells on air-dried collagen gels with fibroblasts or Matrigel. After the culture, lamina densa, which can be detected by a transmission electron microscopy, formed beneath the cells and was used as a new cell culture substrate. On this basement membrane model, primary hepatocytes can survive longer than on a type I collagen matrix and Matrigel. In addition, the cells can maintain their differentiated functions, such as albumin secretion and cytochrome P450 gene expression, on this decellularized basement membrane model at higher levels than cells on a type I collagen matrix and Matrigel [74].

Cell-formed matrices can be used to modify the surfaces of other biomaterials, such as metals. Although titanium has advantageous mechanical properties, titanium alloys are not osteoconductive and have a low affinity for cells. To cope with these problems, surface modification techniques have been developed. To modify titanium surfaces with a native ECM, osteoblast-like SAOS-2 cells were cultured and allowed to synthesise and assemble their ECM on titanium surfaces [76]. After the deposition of ECM proteins, the cell culture surfaces were decellularized to obtain decellularized-matrices-modified titanium surfaces. The number of cells attached to the decellularized-matrices-modified titanium surfaces was greater than found on RGD peptide- or fibronectin-modified titanium surfaces or on bare titanium surfaces. The cells are also able to spread well on the decellularized matrices-modified titanium surfaces.

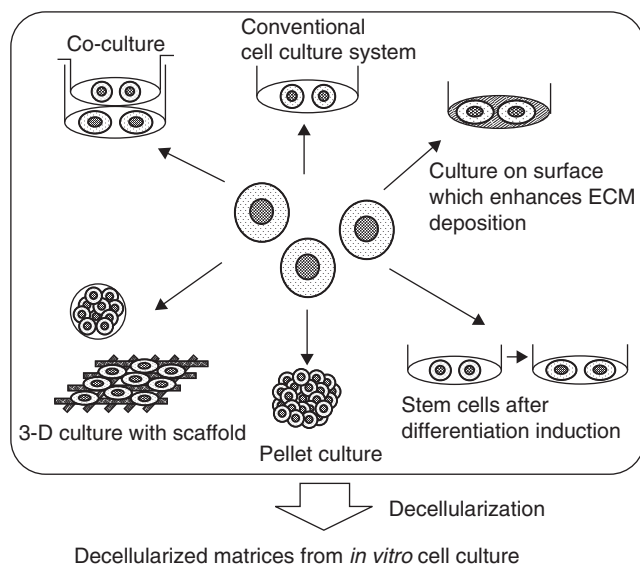


Figure 3. Cell culture methods for the preparation of cell-formed decellularized matrices.

5. Cell-formed decellularized matrices for the regulation of stem cell functions

Stem cells are a concern in tissue engineering due to their capacity for self-renewal and differentiation. To date, there have been a number of efforts to direct stem cell functions. Cell-formed decellularized matrices have been extensively studied for the regulation of stem cell functions. With regard to the regulation of stem cell functions, the maintenance of stemness and the direction of stem cell differentiation are important.

Embryonic stem (ES) cells are usually cultured with feeder cells to maintain their pluripotent differentiation and proliferation potential. The establishment and maintenance of ES cells without feeder cells is one of the most important issues in tissue engineering. To solve this problem, Klimanskaya *et al.* applied decellularized matrices [77]. They prepared decellularized matrices derived from mouse embryonic fibroblasts (or feeder cells). On these matrices, they established new human ES cells from fertilised eggs and found that these ES cells could be maintained on these decellularized matrices in a feeder-free condition with their self-renewal ability and pluripotency intact.

Similar to the approach described above, mesenchymal stem cells can be maintained on decellularized matrices with stemness. When mesenchymal stem cells (MSCs) are cultured *in vitro* on conventional tissue culture plates (TCPs), the differentiation ability of the cells decreases during culture [78]. To avoid this problem, an artificial niche to maintain stemness has been under development. Chen and colleagues developed decellularized matrices derived from MSCs for the maintenance of stemness in the cells [78,79]. On this

decellularized matrix, MSCs can be cultured with the suppression of spontaneous differentiation [79]. Moreover, MSCs can be subcultured on the matrices with their differentiation ability maintained for a longer time than on TCPs [78]. The authors attributed this phenomenon to the regulation of cytokines, such as Wnt ligands and BMPs, by decellularized matrices. They showed that the activity of BMPs was suppressed on the matrices such that spontaneous differentiation was inhibited.

Manipulation of the direction of stem cell differentiation is also an important issue in tissue engineering when using stem cells. There have been several trials to direct stem cell differentiation using decellularized matrices [80-87]. For the differentiation of ES cells or induced pluripotent stem (iPS) cells into pancreatic β cells, Higuchi *et al.* applied a synthetic basement membrane with decellularization [80]. The synthetic basement membrane was prepared from a culture of the HEK293 cell line stably expressing laminin-511. After the deposition of basement membrane components, HEK293 cells were removed to prepare the synthetic basement membrane. On this basement membrane model, mouse ES cells or iPS cells sequentially differentiated into definitive endoderm, pancreatic progenitor cells, and the insulin secreting pancreatic β cells *in vitro*. For chondrogenic differentiation, decellularized 3-dimensional (3D) matrices derived from chondrocytes have been applied [81,82]. Cheng *et al.* developed decellularized matrices derived from chondrocytes cultured in collagen microspheres and evaluated the chondrogenesis of MSCs in the matrices [82]. In these decellularized matrix microspheres, the chondrogenesis of MSCs was supported. Choi *et al.* also developed decellularized matrices derived from chondrocytes [81]. In these decellularized matrices, the chondrogenesis of MSCs was accelerated compared with polyglycolic acid (PGA) scaffolds. Moreover, hypertrophy was suppressed in these scaffolds. Mikos and colleagues developed composite materials of titanium mesh and decellularized matrices derived from osteoblasts and MSC-derived osteoblasts [83,84]. They compared the osteogenesis of MSCs and found that it was accelerated in decellularized matrices with modified titanium mesh compared with bare titanium mesh. Dumas *et al.* prepared the decellularized ECM of osteoblasts cultured under a low-magnitude, high-frequency mechanical stimulation [85]. The decellularized osteogenic matrix promoted the expression of osteoblast markers at the expense of adipogenic markers when MSCs were cultured in the matrix with a multipotential medium.

When stem cells differentiate into somatic cells, the differentiation will proceed in step-by-step manner [88]. During this stepwise differentiation of stem cells, the ECM surrounding differentiating cells is remodelled according to the stage of differentiation [21]. Recently, we have developed decellularized matrices mimicking ECM remodelling during stem cell differentiation, referred to as stepwise tissue-development mimicking matrices [86,87]. We developed the decellularized matrices mimicking the ECM during the osteogenesis or

Table 2. Partial list of cell-formed decellularized matrices.

Cell source	Application	Remarks	Ref.
Fibroblast	Cell culture substrate		[71]
Embryonic fibroblast	Cell culture substrate		[70]
Alveolar epithelial cells	ES cells establishment and their maintenance		[77]
	Basement membrane model	Co-culture with fibroblast	[72]
	Basement membrane model, primary hepatocyte culture, tracheal cell culture	Co-culture with Matrigel	[73-75]
HEK293	Pancreatic lineage differentiation of ES cells	Cells stably expressing laminin-511	[80]
Osteoblast-like SAOS-2	Ti coating for bone implantation		[76]
Mesenchymal stem cell	Maintenance of mesenchymal stem cell		[78,79]
Chondrocyte	Chondrocyte monolayer culture		[98]
Osteogenic mesenchymal stem cells	Scaffold for implantation	ECM formed in a pellet culture	[81,99]
	Chondrogenesis of mesenchymal stem cell	ECM formed in collagen microspheres	[82]
	Osteogenesis of mesenchymal stem cell	In titanium mesh	[83,84]
	Osteogenesis of mesenchymal stem cell	Osteogenic MC3T3-E1	[85]
	Osteogenesis of mesenchymal stem cell	Matrices were prepared in each step of osteogenesis	[86]
Adipogenic mesenchymal stem cell	Adipogenesis of mesenchymal stem cell	Matrices were prepared in each step of adipogenesis	[87]

adipogenesis of MSCs. To prepare the decellularized matrices mimicking ECM, MSCs were induced to undergo osteogenesis or to adipogenesis *in vitro*. At each stage of MSC differentiation, cell-cultured substrates were decellularized and the decellularized matrices were used as stepwise osteogenesis-mimicking matrices or stepwise adipogenesis-mimicking matrices. The osteogenesis of MSC was accelerated in the decellularized matrices mimicking ECM at an early stage compared with those at the undifferentiated stage, and the late stage of osteogenesis, and the bare TCPs [86]. Interestingly, different mechanisms are at work for osteogenesis suppression in the matrices mimicking ECM at the undifferentiated stage and the late stage. In the matrices mimicking ECM at the undifferentiated stage, the osteogenesis was directly inhibited. On the other hand, the osteogenesis was competitively inhibited in the matrices mimicking ECM at the late stage. In the matrices mimicking ECM at the late stage, adipogenesis was unexpectedly observed. However, adipogenesis and the direct suppression of osteogenesis were not observed in the matrices mimicking ECM at the early stage, resulting in the acceleration of osteogenesis. The regulation of the balance between osteogenesis and adipogenesis was due to the regulation of cytokines' activities by the ECM. In the decellularized matrices mimicking ECM at the late stage, β -catenin signal, which can suppress adipogenesis, was suppressed compared with the matrices mimicking ECM at the early stage of osteogenesis.

We have also developed decellularized matrices mimicking ECM during the adipogenesis of MSCs as stepwise adipogenesis-mimicking matrices [87]. Similar to stepwise osteogenesis-mimicking matrices, the adipogenesis of MSCs was accelerated on the matrices mimicking ECM at an early stage of adipogenesis compared with those at the undifferentiated stage and the late stage, and the bare TCPs.

From our research, maturational-stage-specific ECM plays an important role in the direction of stem cell differentiation. Mimicking the ECM at each maturational stage is one of the important approaches to direct stem cell differentiation.

These stepwise tissue-development mimicking matrices will be applied not only to tissue engineering but also to basic biological research. To investigate the role of the ECM during tissue development, protein knocked-down animals are available [89]. Alternatively, surfaces coated with ECM proteins are also used [90]. In the cases using animals, it is difficult to comprehensively investigate the role of the ECM during tissue development. For this purpose, new *in vitro* ECM models mimicking the ECM during tissue development are desired. Stepwise tissue-development mimicking matrices will be one of the good *in vitro* models available to investigate the roles of ECM.

6. Conclusions

Insight into the microstructure and function of *in vivo* ECM helps the understanding of the necessity for ECM scaffolds and substrates for tissue engineering. Various kinds of ECM scaffolds and substrates have been prepared from simply structured tissues and whole organs with complicated structures. ECM scaffolds can also be prepared from cultured cells so that there is no problem with donor tissue availability and cells at different development stages can be controlled. The ECM scaffolds and substrates have been used to control stem cell functions and the regeneration of various types of tissues and organs. Decellularized matrices have been used for stemness maintenance as a stem cell niche and for the direction of stem cell differentiation. In addition to applications for tissue engineering, the decellularized matrices will be good *in vitro* ECM models. Therefore, the decellularized

matrices will be useful matrices for not only tissue engineering and biological therapy but also for basic biological research.

7. Expert opinion

Decellularized matrices derived from tissues/organs and cultured cells are one of the useful matrices for tissue engineering and stem cell manipulation as described above. To use natural ECM proteins, most of the research has been focused on isolated ECM proteins and their combinations, although the ECM has a complex composition in each specific tissue [11,12,90-93]. Instead of the application of isolated ECM proteins, Matrigel, an extract of basement membrane secreted from Engelbreth-Holm-Swarm sarcoma cells, has also been used [94,95]. As evidenced by the studies using Matrigel and its combination with other isolated proteins, the full ECM composition is required to exert maximal functions [92-95]. In addition, the composition of the ECM depends on the types of cells, tissues and organs [8], and only native-tissue-like matrices can induce the highest effect on cellular functions [20]. Furthermore, the microstructure and biomechanical properties of the ECM facilitate functional tissue engineering and the engineered tissues and organs can replicate the function immediately after implantation [30,40-42,55]. Given these factors, decellularized matrices are useful for tissue engineering and comprehensive study of ECM.

Years ago, decellularization was focused on simple tissues such as small intestinal submucosa, amniotic membrane, skin, heart valves and blood vessels [31-39,44,45,59,63-65]. In recent years, with the development of new and advanced decellularization methods, the decellularization of complicated organs, such as the heart, liver and lung, has become realistic [30,40-42,55]. The intricate blood vessel and airway networks in these complicated organs are almost impossible to build up by conventional technology. The strong desire for the regeneration of complicated organs, such as the heart, liver, lung and pancreas, has pushed the development of ECM scaffolds. The development of a decellularized matrix for large and complicated tissues and organs will continue as the popular trend in tissue engineering and scaffolding research.

Although the decellularized tissue- and organ-derived matrices are similar to the *in vivo* ECM, they cannot mimic the development process of tissue or body. To mimic the remodelling of the ECM during development, the use of stem cells that are controlled at different stages of development is important. Theoretically, the preparation of ECM mimicking the whole development process is possible if embryonic stem cells or induced pluripotent cells are used.

The advantages of the decellularized ECM are evident. There are also some problems and challenges. One of the problems is the variation of the ECM from different donors. The subtle or evident difference of decellularized ECM scaffolds is hardly eliminated because each of the donor tissues and organs is from different individuals. To solve this problem, it is reasonable to consider usage of synthetic

matrices including synthetic peptides such as RGD and proteins such as collagen. However, it is difficult for the synthetic matrices to induce full cellular functions as native ECMs are composed of several domains [96] and complicated molecule networks [4] and have synergistic effects. Another problem of decellularized ECM is the availability of donor tissues and organs. Xenogeneic and allogeneic tissues and organs have been frequently used because of their availability. However, there is a problem with inflammatory and immunological reactions [38,97]. Xenogeneic and allogeneic cellular antigens can be recognised by the host and induce inflammatory and immunological reactions, and therefore result in the rejection of implanted tissues and organs. Because ECM components are well-conserved among species and tolerated even by xenogeneic recipients, the host responses can be minimised if the cellular components are completely removed. Therefore the development of a decellularization method to completely remove cellular components is important to help solve the problem of inflammatory and immunological reactions. The development of autologous ECM scaffolds that are derived from autologous tissues and organs is the optimal method to solve this problem. However, it is almost impossible to use autologous tissues and organs for decellularization. The use of cultured cells to prepare autologous scaffolds should be considered as autologous cells are available and can be expanded by *in vitro* cell culture.

Compared with decellularized-tissue- and organ-derived ECM scaffolds, cell-formed decellularized matrices have the advantages of unlimited availability and adaptability for different developmental stages. There are also some problems with the cell-formed decellularized matrices. It is difficult to mimic the native ECM-like complicated architectures. And the ECM secretion pattern of *in vitro* cultured cells is different from that of *in vivo* cells. Therefore, the possibility of difference of ECM composition and structure between cell-formed decellularized matrices and native ECMs should be considered when the cell-formed decellularized matrices are used.

Finally, besides the scientific and technical bases of decellularized matrices, regulatory issues should also be considered to facilitate their clinical applications. Regulation of production, management of donor tissues and organs, effectiveness and safety evaluation, quality control, application protocols and guidelines, standardization and ethics are critical issues for the development and commercialization of decellularized ECM scaffolds.

Despite these problems and challenges, the progression of research into decellularized matrices will open the way for the research and development of new biological therapies, such as engineered tissues for tissue transplantation.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

1. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920-6
2. Griffith LG, Naughton G. Tissue engineering-current challenges and expanding opportunities. *Science* 2002;295:1009-14
3. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffolds material: structure and function. *Acta Biomater* 2009;5:1-13
4. Adachi E, Hopkinson I, Hayashi T. Basement-membrane stromal relationships: interactions between collagen fibrils and the lamina densa. *Int Rev Cytol* 1997;173:73-156
5. Giancotti FG, Ruoslahti E. Integrin signaling. *Science* 1999;285:1028-32
6. Rahman S, Patel Y, Murray J, et al. Novel hepatocyte growth factor (HGF) binding domains on fibronectin and vitronectin coordinate a distinct and amplified Met-integrin induced signalling pathway in endothelial cells. *BMC Cell Biol* 2005;6:8
7. Comoglio PM, Boccaccio C, Trusolino L. Interactions between growth factor receptors and adhesion molecules: breaking the rules. *Curr Opin Cell Biol* 2003;15:565-71
8. Manabe R, Tsutsui K, Yamada T, et al. Transcriptome-based systematic identification of extracellular matrix proteins. *Proc Natl Acad Sci USA* 2008;105:12849-54
9. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 1994;124:619-26
10. Grossmann J. Molecular mechanisms of "detachment-induced apoptosis-Anoikis". *Apoptosis* 2002;7:247-60
11. Gu J, Fujibayashi A, Yamada KM, Sekiguchi K. Laminin-10/11 and fibronectin differentially prevent apoptosis induced by serum removal via phosphatidylinositol 3-kinase/Akt- and MEK1/ERK-dependent pathways. *J Biol Chem* 2002;277:19922-8
12. Gu J, Sumida Y, Sanzen N, Sekiguchi K. Laminin-10/11 and fibronectin differentially regulate integrin-dependent Rho and Rac activation via p130Cas-CrkII-Dock180 pathway. *J Biol Chem* 2001;276:27090-7
13. Serandour A-L, Loyer P, Garnier D, et al. TNFalpha-mediated extracellular matrix remodeling is required for multiple division cycles in rat hepatocytes. *Hepatology* 2005;41:478-86
14. Mohammed FF, Pennington CJ, Kassiri Z, et al. Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. *Hepatology* 2005;41:857-67
15. Gutierrez J, Osses N, Brandan E. Changes in secreted and cell associated proteoglycan synthesis during conversion of myoblasts to osteoblasts in response to bone morphogenetic protein-2: role of decorin in cell response to BMP-2. *J Cell Physiol* 2006;206:58-67
16. Moreno M, Munoz R, Aroca F, et al. Biglycan is a new extracellular component of the Chordin-BMP4 signaling pathway. *EMBO J* 2005;24:1397-405
17. Kerever A, Schnack J, Vellinga D, et al. Novel extracellular matrix structures in the neural stem cell niche capture the neurogenic factor fibroblast growth factor 2 from the extracellular milieu. *Stem Cells* 2007;25:2146-57
18. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;126:677-89
19. Alcaraz J, Xu R, Mori H, et al. Laminin and biomimetic extracellular elasticity enhance functional differentiation in mammary epithelia. *EMBO J* 2008;27:2829-38
20. Sellaro TL, Ravindra AK, Stolz DB, Badylak SF. Maintenance of hepatic sinusoidal endothelial cell phenotype in vitro using organ-specific extracellular matrix scaffolds. *Tissue Eng* 2007;13:2301-10
21. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. *J Cell Sci* 2008;121:255-64
22. Midwood KS, Williams LV, Schwarzbauer JE. Tissue repair and the dynamics of the extracellular matrix. *Int J Biochem Cell Biol* 2004;36:1031-7
23. Bedossa P, Paradis V. Liver extracellular matrix in health and disease. *J Pathol* 2003;200:504-15
24. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials* 2006;27:3675-83
25. Jackson DW, Grood ES, Cohn BT, et al. The effects of in situ freezing on the anterior cruciate ligament. An experimental study in goats. *J Bone Joint Surg Am* 1991;73:201-13
26. Yoo JJ, Meng J, Oberpenning F, Atala A. Bladder augmentation using allogenic bladder submucosa seeded with cells. *Urology* 1998;51:221-5
27. Chen F, Yoo JJ, Atala A. Acellular collagen matrix as a possible "off the shelf" biomaterial for urethral repair. *Urology* 1999;54:407-10
28. Woods T, Gratzner PF. Effectiveness of three extraction techniques in the development of a decellularized bone-anterior cruciate ligament-bone graft. *Biomaterials* 2005;26:7339-49
29. McFetridge PS, Daniel JW, Bodamyali T, et al. Preparation of porcine carotid arteries for vascular tissue engineering applications. *J Biomed Mater Res* 2004;70A:224-34
30. Ott HC, Matthiesen TS, Goh S-K, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med* 2008;14:213-21
31. Steinhoff GU, Stock N, Karim H, et al. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits in vivo restoration of valve tissue. *Circulation* 2000;102:III-50-5
32. Bertipaglia BF, Ortolani L, Petrelli G, et al. Cell characterization of porcine aortic valve and decellularized leaflets repopulated with aortic valve interstitial cells: the VESALIO Project (Vitalitate Exornatum Succedaneum Aorticum Labore Ingenioso Obtenibitur). *Ann Thorac Surg* 2003;75:1274-82
33. Tedder ME, Simionescu A, Chen J, et al. Assembly and testing of stem cell-seeded layered collagen constructs for heart valve tissue engineering. *Tissue Eng A*: published online 6 September 2010, doi:10.1089/ten.tea.2010.0138
34. Schmidt CE, Baier JM. A cellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. *Biomaterials* 2000;21:2215-31

35. Singelyn JM, DeQuach JA, Seif-Naraghi SB, et al. Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering. *Biomaterials* 2009;30:5409-16
36. Badylak SF, Lantz GC, Coffey A, Geddes LA. Small intestinal submucosa as a large diameter vascular graft in the dog. *J Surg Res* 1989;47:74-80
37. Funamoto S, Nam K, Kimura T, et al. The use of high-hydrostatic pressure treatment to decellularized blood vessels. *Biomaterials* 2010;31:3590-5
38. Badylak SF. The extracellular matrix as a biologic scaffold material. *Biomaterials* 2007;28:3587-93
39. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. *Acta Biomater* 2009;5:1-13
40. Ott HC, Clippinger B, Conrad C, et al. Regeneration and orthotopic transplantation of a bioartificial lung. *Nat Med* 2010;16:927-33
41. Petersen TH, Calle EA, Zhao L, et al. Tissue-engineered lungs for in vivo implantation. *Science* 2010;329:538-41
42. Price AP, England KA, Matson AM, et al. Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. *Tissue Eng A* 2010;16:2581-91
43. Jungebluth P, Go T, Asnaghi A, et al. Structural and morphologic evaluation of a novel detergent-enzymatic tissue-engineered tracheal tubular matrix. *J Thorac Cardiovasc Surg* 2009;138:586-93
44. Wainwright D, Madden M, Luterma A, et al. Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. *J Burn Care Rehabil* 1996;17:124-36
45. Stern M. The grafting of preserved amniotic membrane to burned and ulcerated surfaces, substituting skin grafts. *JAMA* 1913;60:973-4
46. Hudson TW, Liu SY, Schmidt CE. Engineering an improved acellular nerve graft via optimized chemical processing. *Tissue Eng* 2004;10:1346-58
47. Sondell M, Lundborg G, Kanje M. Regeneration of the rat sciatic nerve into allografts made acellular through chemical extraction. *Brain Res* 1998;795:44-54
48. Choi JS, Williams JK, Margaret G, et al. Bioengineering endothelialized neo-corneas using donor-derived corneal endothelial cells and decellularized corneal stroma. *Biomaterials* 2010;31:6738-45
49. Sasaki S, Funamoto S, Hashimoto Y, et al. In vivo evaluation of a novel scaffold for artificial corneas prepared by using ultrahigh hydrostatic pressure to decellularized porcine corneas. *Mol Vis* 2009;15:2022-8
50. Hashimoto Y, Funamoto S, Sasaki S, et al. Preparation and characterization of decellularized cornea using high-hydrostatic pressurization for corneal tissue engineering. *Biomaterials* 2010;31:3941-8
51. Kajitani M, Wadia Y, Hinds MT, et al. Successful repair of esophageal injury using an elastin based biomaterial patch. *ASAIO J* 2001;47:342-5
52. Ozeki M, Narita Y, Kagami H, et al. Evaluation of decellularized esophagus as a scaffold for cultured esophageal epithelial cells. *J Biomed Mater Res* 2006;79A:771-8
53. Reid LM, Rojkind M. New techniques for culturing differentiated cells: reconstituted basement membrane rafts. *Methods Enzymol* 1978;58:263-78
54. Rojkind M, Gattmaitan Z, Mackensen S, et al. Connective tissue biomatrix: its isolation and utilization for long-term cultures of normal rat hepatocytes. *J Cell Biol* 1980;87:255-63
55. Uygun BE, Soto-Gutierrez A, Yagi H, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med* 2010;16:814-20
56. Nakayama KH, Batchelder CA, Lee CI, Tarantal AF. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. *Tissue Eng A* 2010;16:2207-16
57. Yang B, Zhang Y, Zhou L, et al. Development of a porcine bladder acellular matrix with well-preserved extracellular bioactive factors for tissue engineering. *Tissue Eng C* 2010;16:1201-11
58. Stapleton TW, Ingram J, Katta J, et al. Development and characterization of an acellular porcine medial meniscus for use in tissue engineering. *Tissue Eng A* 2008;14:505-18
59. Uriel S, Huang J, Moya ML, et al. The role of adipose protein derived hydrogels in adipogenesis. *Biomaterials* 2008;29:3712-19
60. Flynn LE. The use of decellularized adipose tissue to provide an inductive microenvironment for the adipogenic differentiation of human adipose-derived stem cells. *Biomaterials* 2010;31:4715-24
61. Abberton KM, Bortolotto SK, Woods AA, et al. Myogel, a novel, basement membrane-rich, extracellular matrix derived from skeletal muscle, is highly adipogenic in vivo and in vitro. *Cells Tissues Organs* 2008;188:347-58
62. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. *Am J Obstet Gynecol* 1979;134:833-45
63. Wilshaw S-P, Kearney JN, Fisher J, Ingham E. Production of an acellular amniotic membrane matrix for use in tissue engineering. *Tissue Eng* 2006;12:2117-29
64. Hao Y, Ma DH, Hwang DG, et al. Identification of antiangiogenic and anti-inflammatory proteins in human amniotic membrane. *Cornea* 2000;19:348-52
65. Kjaergaard N, Hein M, Hyttel L, et al. Antibacterial properties of human amnion and chorion in vitro. *Eur J Obstet Gynecol Reprod Biol* 2001;94:224-9
66. Nakajima I, Yamaguchi T, Ozutsumi K, Aso H. Adipose tissue extracellular matrix: newly organized by adipocytes during differentiation. *Differentiation* 1998;63:193-200
67. Freytes DO, Martin J, Velankar SS, et al. Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. *Biomaterials* 2008;29:1630-7
68. Grinnell F, Feld MK. Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum-containing medium. *J Biol Chem* 1982;257:4888-93
69. Hoshiba T, Cho CS, Murakawa A, et al. The effect of natural extracellular matrix deposited on synthetic polymers on cultured primary hepatocytes. *Biomaterials* 2006;27:4519-28

70. Chen LB, Murray A, Segal RA, et al. Studies on intercellular LETS glycoprotein matrices. *Cell* 1978;14:377-91
71. Hedman K, Kurkinen M, Alitalo K, et al. Isolation of the pericellular matrix of human fibroblast culture. *J Cell Biol* 1979;81:83-91
72. Furuyama A, Kimata K, Mochitate K. Assembly of basement membrane in vitro by cooperation between alveolar epithelial cells and pulmonary fibroblasts. *Cell Struct Funct* 1997;22:603-14
73. Furuyama A, Mochitate K. Assembly of the exogenous extracellular matrix during basement membrane formation by alveolar epithelial cells in vitro. *J Cell Sci* 2000;113:859-68
74. Hoshiba T, Mochitate K, Akaike T. Hepatocytes maintain their function on basement membrane formed by epithelial cells. *Biochem Biophys Res Commun* 2007;359:151-6
75. Hosokawa T, Betsuyaku T, Nishimura M, et al. Differentiation of tracheal basal cells to ciliated cells and tissue reconstruction on the synthesized basement membrane substratum in vitro. *Connect Tissue Res* 2007;48:9-18
76. Pham MT, Reuther H, Maitz MF. Native extracellular matrix coating on Ti surfaces. *J Biomed Mater Res* 2003;66A:310-16
77. Klimanskaya I, Chung Y, Meisner L, et al. Human embryonic stem cells derived without feeder cells. *Lancet* 2005;365:1636-41
78. Lai Y, Sun Y, Skinner CM, et al. Reconstitution of marrow-derived extracellular matrix ex vivo: a robust culture system for expanding large-scale highly functional human mesenchymal stem cells. *Stem Cells Dev* 2010;19:1095-107
79. Chen X-D, Dusevich V, Feng JQ, et al. Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived mesenchymal progenitor cells and prevents their differentiation into osteoblasts. *J Bone Miner Res* 2007;22:1943-56
80. Higuchi Y, Shiraki N, Yamane K, et al. Synthesized basement membranes direct the differentiation of mouse embryonic stem cells into pancreatic lineages. *J Cell Sci* 2010;123:2733-42
81. Choi K-H, Choi BH, Park SR, et al. The chondrogenic differentiation of mesenchymal stem cells on an extracellular matrix scaffold derived from porcine chondrocytes. *Biomaterials* 2010;31:5355-65
82. Cheng H-W, Tsui Y-K, Cheung KMC, et al. Decellularization of chondrocyte-encapsulated collagen microspheres: a three-dimensional model to study the effects of acellular matrix on stem cell fate. *Tissue Eng C* 2009;15:697-706
83. Datta N, Pham QP, Sharma U, et al. In vitro generated extracellular matrix and fluid shear stress synergistically enhance 3D osteoblastic differentiation. *Proc Natl Acad Sci USA* 2006;103:2488-93
84. Pham QP, Kasper FK, Baggett LS, et al. The influence of an in vitro generated bone-like extracellular matrix on osteoblastic gene expression of marrow stromal cells. *Biomaterials* 2008;29:2729-39
85. Dumas V, Ducharme B, Perrier A, et al. Extracellular matrix produced by osteoblasts cultured under low-magnitude, high-frequency stimulation is favourable to osteogenic differentiation of mesenchymal stem cells. *Calcif Tissue Int* 2010;87:351-64
86. Hoshiba T, Kawazoe N, Tateishi T, Chen G. Development of stepwise osteogenesis-mimicking matrices for the regulation of mesenchymal stem cell functions. *J Biol Chem* 2009;284:31164-73
87. Hoshiba T, Kawazoe N, Tateishi T, Chen G. Development of extracellular matrices mimicking stepwise adipogenesis of mesenchymal stem cells. *Adv Mater* 2010;22:3042-7
88. D'Amour KA, Bang AG, Eliazar S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006;24:1392-401
89. Xu T, Bianco P, Fisher LW, et al. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nat Genet* 1998;20:78-82
90. Spiegelman BM, Ginty CA. Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes. *Cell* 1983;35:657-66
91. Brodtkin KR, Garcia AJ, Levenston ME. Chondrocyte phenotypes on different extracellular matrix monolayers. *Biomaterials* 2004;25:5929-38
92. Yi JY, Yoon YH, Park HS, et al. Reconstruction of basement membrane in skin equivalent; role of laminin-1. *Arch Dermatol Res* 2001;293:356-62
93. Nagata NA, Inoue K, Tabata Y. Co-culture of extracellular matrix suppresses the cell death of rat pancreatic islets. *J Biomater Sci Polym Ed* 2002;13:579-90
94. Oda H, Nozawa K, Hitomi Y, Kakinuma A. Laminin-rich extracellular matrix maintains high level of hepatocyte nuclear factor 4 in rat hepatocyte culture. *Biochem Biophys Res Commun* 1995;212:800-5
95. Barcellos-Hoff MH, Aggeler J, Ram TG, Bissell MJ. Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development* 1989;105:223-35
96. Bork P, Downing AK, Kieffer B, Campbell ID. Structure and distribution of modules in extracellular proteins. *Q Rev Biophys* 1996;29:119-67
97. Kasimir MT, Rieder E, Seebacher G, et al. Decellularization does not eliminate thrombogenicity and inflammatory stimulation in tissue-engineered porcine heart valves. *J Heart Valve Dis* 2006;15:278-86
98. Dessau W, Vertel BM, von der Mark H, von der Mark K. Extracellular matrix formation by chondrocytes in monolayer culture. *J Cell Biol* 1981;90:78-83
99. Jin CZ, Park SR, Choi BH, et al. In vivo cartilage tissue engineering using a cell-derived extracellular matrix scaffold. *Artif Organs* 2007;31:183-92

Affiliation

Takashi Hoshiba PhD, Hongxu Lu PhD, Naoki Kawazoe PhD & Guoping Chen[†] PhD
[†]Author for correspondence
National Institute for Materials Science, Biomaterials Center, Tsukuba, Ibaraki 305-0044, Japan
Tel: +81 29 860 4496; Fax: +81 29 860 4714; E-mail: Guoping.Chen@nims.go.jp