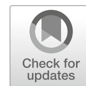
Chapter 2
Polymeric and Biomimetic ECM Scaffolds for Tissue Engineering Applications



Guoping Chen and Naoki Kawazoe

Abstract Porous scaffolds can provide temporary biomimetic microenvironments to control cell functions and to guide new tissue regeneration. Many methods have been developed to prepare porous scaffolds of biodegradable polymers and acellular extracellular matrix (ECM) for tissue engineering applications. Ice particulate method and sacrificial template method have been used to prepared scaffolds with well-controlled pore structures such as open surface pores and interconnected bulk pores for easy cell seeding, migration, and distribution. Porous scaffolds with interconnected pore structure, funnel-like structure, and micropatterned structures have been prepared by these methods. Composite scaffolds of biodegradable synthetic polymers and naturally derived polymers have been prepared by hybridization method to combine the advantages of each type of polymers. Furthermore, cell-derived biomimetic ECM scaffolds have been prepared by cell culture method. Composition of the ECM scaffolds can be adjusted by using different type of cells or controlling the differentiation of stem cells. This chapter features and summarizes the details of these methods and scaffolds.

Keywords Polymer scaffold · Porous scaffold · Biodegradable polymer · Synthetic polymer · Naturally derived polymer · Pore structure · Funnel-like structure · ECM scaffold · Biomimetic · Micropatterned structure · Tissue engineering

2.1 Introduction

Polymeric and biomimetic scaffolds have been a broad application in tissue engineering to control cell functions and to provide temporary support for the regeneration of functional new tissues and organs [1–3]. The scaffolds can be prepared from biodegradable polymers, either synthetic or naturally derived. The most frequently

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used biodegradable synthetic polymers for tissue engineering are aliphatic polyesters, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic acid-*co*-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL). Naturally derived polymers are produced from living organisms and can be categorized as proteins, polysaccharides, polyhydroxyalkanoates, and polynucleotides. The first two categories are usually used to prepare porous scaffolds, which are usually modified or cross-linked to control their degradation to support cell culture and tissue formation. Acellular matrices are also very useful scaffolds for tissue engineering because of their similarity to the in vivo microenvironments [4, 5].

Scaffolds can be prepared by many methods, such as particle leaching, freeze-drying, phase separation, gas foaming, electrospinning, fiber bonding, and 3D printing [6]. The nano- and microstructures of scaffolds can be tailored for specific tissue engineering applications. The physical and biochemical properties of scaffolds can affect the functions of cells cultured in vitro or in vivo. Porous scaffolds with a variety of nano- and microstructure, mechanical property, and biochemical composition have been prepared by these methods.

Scaffold properties have diverse influences on cell functions. The pore structure of scaffolds can affect the cell behaviors, such as distribution, migration, assembly, and tissue formation [7, 8]. Open pore structure of scaffolds is the premise to enable homogeneous cell seeding and homogenous tissue formation. Scaffolds with isotropic and open pore structure enable a homogeneous distribution of cells and formation of a homogenous tissue.

Chemical composition of scaffolds can also greatly affect cell functions. Cell adhesion and spreading are usually more promoted by naturally derived polymer scaffolds compared with synthetic polymer scaffolds. Cells in normal tissue are surrounded with ECM which serves as a substrate to modulate cell behaviors. ECM of normal tissue has multiple components, such as collagen, laminin, aggrecan, hyaluronic acid, and fibronectin. Scaffolds with a similar composition to that of normal tissue ECM should benefit cell proliferation and tissue formation [9]. This chapter introduces the methods to prepare polymer porous scaffolds with well-controlled microporous structures, hybrid scaffolds of biodegradable synthetic polymers and naturally derived polymers, and cell-derived biomimetic ECM scaffolds.

2.2 Scaffolds Prepared with Free Ice Particulates

Scaffolds used for tissue engineering should have an adequate microporous structure for enabling cellular penetration into the construct to obtain a desirable cell distribution. Although many three-dimensional porous scaffolds have been developed from biodegradable polymers, their pore structures should be controlled to make their surface pores open and bulk pores interconnected. When porous scaffolds are used for cell seeding and 3D cell culture, cells are easily allocated and distributed in the peripheral areas which results in nonhomogeneous cell distribution and partial

tissue formation in the outermost peripheral layers of the scaffolds. Open pore structure is required to guarantee smooth entry of cells into the inner pores of the scaffolds during cell seeding. Meanwhile, pore interconnectivity is required to allow cells for free movement to reach all the pores throughout the scaffolds.

There are a few methods that have been developed for controlling various aspects of the pore structures, such as pore size, porosity, and interconnectivity of the scaffolds [10–13]. Among these methods, the porogen-leaching method offers many advantages for the easy manipulation and control of pore size and porosity. In this method, the porogen materials can leave replica pores after leaching. Selection of porogen materials is important to decide the pore structures. Isolated particles of porogen materials may result in the formation of isolated pores, a situation which is not desirable for tissue engineering scaffolds. To improve pore interconnectivity, the porogen materials are bonded before mixing them with polymer matrix [14, 15]. However, the bonded porogen materials require organic solvents for leaching and the residual solvents are toxic to cells. Mixing of polymer solution with the bonded porogen materials becomes difficult if the polymer solution has a high viscosity. To overcome these problems, an approach using free ice particulates as a porogen material has been developed [11, 16, 17]. Many porous scaffolds and their composites have been prepared by this method [18–27].

In the ice particulate method, free ice particulates are at first prepared. Free ice particulates can be easily prepared by spraying or injecting water into liquid nitrogen through a sprayer or capillary. Free ice particulates formed by spraying method are spherical. Their diameters can be controlled by the spraying speed. The ice particulates can be sieved by sieves with different mesh pores under low temperatures to obtain ice particulates with desired diameters. Subsequently, the free ice particulates are homogenously mixed with polymer solution. The mixing temperature is set at a temperature where the ice particulates do not melt and polymer solution does not freeze. Finally, the mixture is frozen and freeze-dried to form porous structures. Ice particulates can be easily and completely removed by freeze-drying. The porous scaffolds are cross-linked after freeze-drying if the polymers are water soluble. During the preparation procedures, the pre-prepared free ice particulates not only work as porogens to control the pore size and porosity, but also work as nuclei to initiate the formation of new ice crystals during freezing process if polymer aqueous solution is used. Pore structure is decided by both the free ice particulates and the newly formed ice crystals. The newly formed ice crystals can increase the pore interconnectivity if they grow and extend from the pre-prepared free ice particulates.

Collagen porous scaffolds have been prepared by this method [11]. Free ice particulates having diameters of 150–250, 250–355, 355–425, and 425–500 μm are used to control the bulk pore structures of collagen scaffolds. Gross appearance and microstructures of the collagen porous scaffolds prepared with 2 (w/v)% collagen and free ice particulates at a ratio of 50:50 (w/v, ice particulates/collagen solution) are shown in Fig. 2.1. The collagen porous scaffolds have large spherical pores and small pores. The small pores surround the large spherical pores, and are located on the walls of large pores. The large spherical pores are evenly distributed and well stacked. The small pores on the walls of large pores connect the large pores,

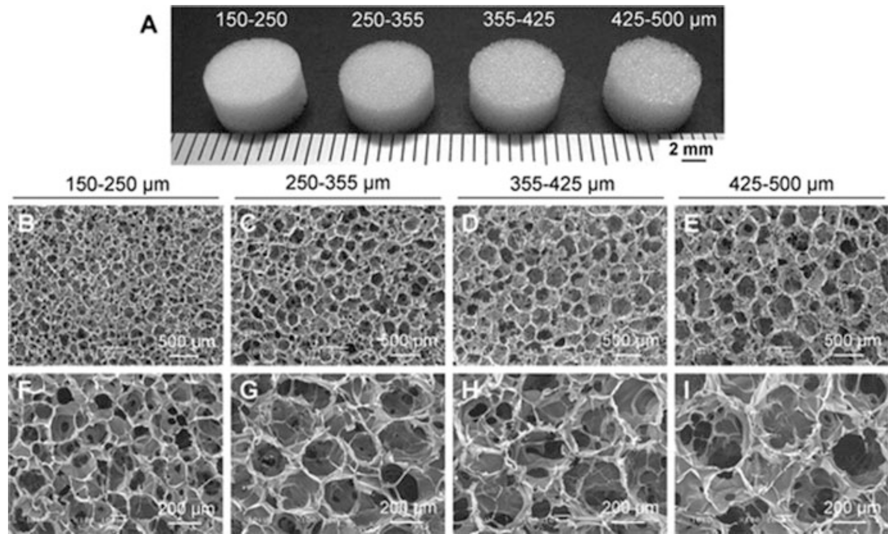

Fig. 2.1 Gross appearances (**a**) and SEM micrographs (**b**–**i**) of the cross-sections of four types of collagen porous scaffolds prepared with ice particulates having diameter ranges of 150–250 (**b**, **f**), 250–355 (**c**, **g**), 355–425 (**d**, **h**), and 425–500 μm (**e**, **i**) at low (**b**–**e**) and high (**f**–**i**) magnifications. (Adapted from Ref. [11] with permission from Elsevier)

making the scaffold well interconnected. The size and density of large pores are dependent on the size and ratio of free ice particulates used to prepare the scaffolds because they are the negative replicas of the free ice particulates. The small pores are the negative replicas of ice crystals that are formed during freezing, which size is dependent upon the freezing temperature. When the collagen porous scaffolds are used for culture of bovine articular chondrocytes, cells can be easily seeded and homogenously distributed throughout the scaffolds. The homogenous cell distribution in the four types of collagen porous scaffolds should be due to the good interconnectivity of the scaffolds. The interconnectivity among the spherical large pores facilitates the smooth delivery of cells in the scaffolds to each corner of the scaffolds.

The ratio of ice particulates and collagen concentration has some influence on the pore structure and mechanical property of collagen porous scaffolds. When collagen porous scaffolds prepared with 25, 50, and 75 (v/w)% ice particulates having a diameter from 335 to 425 μm are compared, the large spherical pores in the scaffolds prepared with 25 (v/w)% ice particulates are sparsely distributed. When 75% ice particulates are used, some collapsed large pores are observed. With a high ratio of ice particulates, the collagen aqueous solution filling the spaces between the spherical ice particulates decreases and the collagen matrix surrounding the large pores decreases. In addition, mixing of the ice particulates and the collagen aqueous solution becomes difficult when the ice particulate ratio is high. The collapsed large pores can be due to the less dense collagen matrix and incomplete mixing.

Collagen scaffolds prepared with 50 (w/v)% ice particulates have the most homogenous pore structure.

The effect of the collagen concentration on the pore structure is investigated by fixing the ice particulate ratio at 50% (w/v) and changing the collagen concentration from 1% to 3% (w/v). Collapsed large pores are observed in the collagen scaffolds prepared with 1% and 3% collagen aqueous solutions. The collapsed large pores in collagen scaffolds prepared with the 1% collagen aqueous solution may be because of the low concentration which results in a less dense collagen matrix surrounding the large pores. The case involving the 3% collagen aqueous solution may be due to incomplete mixing because the 3% collagen solution is too viscous. The collagen scaffold prepared with 2% collagen solution has the most homogeneous pore structure.

When collagen concentration is fixed at 2% and the ratio of ice particulates is changed, the Young's modulus of collagen porous scaffolds increases in the following order: 75% < 25% < 50%. The collagen porous scaffolds prepared with 50% ice particulates have the highest Young's modulus. The difference in the mechanical properties is mainly ascribed to the different pore structures. The spherical pores formed by ice particulates are thought to resist mechanical loading, therefore reinforcing the collagen scaffolds. The high mechanical strength of the collagen scaffolds prepared with 50% ice particulates should be due to the most appropriate packing of the large spherical pores and appropriate filling of the collagen matrix between the large spherical pores. The low mechanical strength of the collagen scaffold prepared with 75% ice particulates may be due to the partially collapsed large pore structure. When the ratio of ice particulates is fixed at 50 (w/v)%, the Young's modulus increases as the collagen concentration increases. A dense collagen matrix surrounding the large pores can be formed to reinforce the scaffolds when the collagen concentration increases.

The ice particulate method has also been used to prepare porous scaffolds of gelatin, and hyaluronic acid/collagen [19, 20]. This method is applicable for many naturally derived polymers. Most of the naturally derived polymers are water soluble. There are many advantages of free ice particulate method for scaffold preparation of naturally derived polymers because the method is proceeded at low temperature and no organic solvent is used. The method is good for incorporation of growth factors in the porous scaffolds, while maintaining their bioactivities.

The method can also be used for scaffold preparation of biodegradable synthetic polymers [18]. Synthetic polymers are dissolved in organic solvents that have a much lower freezing temperature than the melting temperature of the ice particulates. The temperature of biodegradable polymer solution can be decreased to avoid melting of ice particulates during mixing of synthetic polymer solution and the free ice particulates. Freezing of the mixture can induce phase separation of synthetic polymer solution among the ice particulates, resulting in the formation of microporous wall after freeze-drying. However, the mechanical property of biodegradable synthetic polymer scaffolds prepared by this method is much lower than the scaffolds prepared by normal porogen-leaching method using salt particles or sugar particles.

2.3 Funnel-Like Porous Scaffolds and Micropatterned Porous Scaffolds Prepared with Embossing Ice Particulates

To make the scaffold surface pores open, a method using embossing ice particulates has been used [28]. The method is similar to the free ice particulate method. The ice particulates are formed on a surface, and then used as a template to prepare porous scaffolds. As a general procedure, water droplets are at first formed on a thin film by spraying or injecting water, or applying moisture on a hydrophobic surface. The size of the water droplets can be controlled by the number of spraying times, injected water volume, or the moisture application time. Embossing ice particulates are formed after freezing the water droplets. And then, the embossing ice particulates are used as a template to prepare porous scaffolds. The freezing, freeze-drying, cross-linking, and washing steps during scaffold preparation are the same as those of the above-mentioned procedures of free ice particulate method. An aqueous solution of naturally derived polymers is eluted onto the embossing ice particulates, and the construct is frozen. The frozen construct is freeze-dried to remove the embossing ice particulates and ice crystals that are newly formed during freezing. Porous scaffolds having open surface pore structures are prepared after cross-linking and washing.

The method has been used to prepare porous scaffolds of collagen, chitosan, hyaluronic acid, and glycosaminoglycan that have open surface pore structures [29–31]. The porous scaffolds have large open pores on their surfaces and small pores underlying the large surface pores. Such a structure likes a funnel, and therefore the porous scaffolds are referred as funnel-like porous scaffolds. The morphology, size and density of large surface pores are dependent on the embossing ice particulates because they are the negative replicas of the embossing ice particulates. The small pores are the negative replicas of ice crystals that are newly formed during freezing. The size of small pores is dependent on the freezing temperature as mentioned above.

The embossing ice particulate method can be used to prepare micropatterned pore structures in porous scaffolds [32]. In such an application, the embossing ice particulates are micropatterned. Micropatterned ice particulates or ice lines are at first prepared, and used as templates to prepare the micropatterned porous scaffolds. The micropatterned ice particulates or ice lines are prepared by ejecting water droplets through a dispensing machine at a low temperature. By designing an ejection program, the micropatterns can be tailored. Figure 2.2a–h shows some micropatterns of ice particulates and the respective micropatterned collagen porous scaffolds. The micropatterned pore layers can be stacked to construct collagen porous scaffolds with 3D micropatterned pores (Fig. 2.2i, j).

To prepare the 3D micropattern pore structures, polymer solution is eluted on the micropatterned ice particulates that are formed on a film (first layer of ice particulates) and frozen. The frozen polymer solution on the first layer of micropatterned ice particulates is used to prepare the second layer of micropatterned ice particulates (second layer of ice particulates) instead of the film. Polymer solution is eluted on the

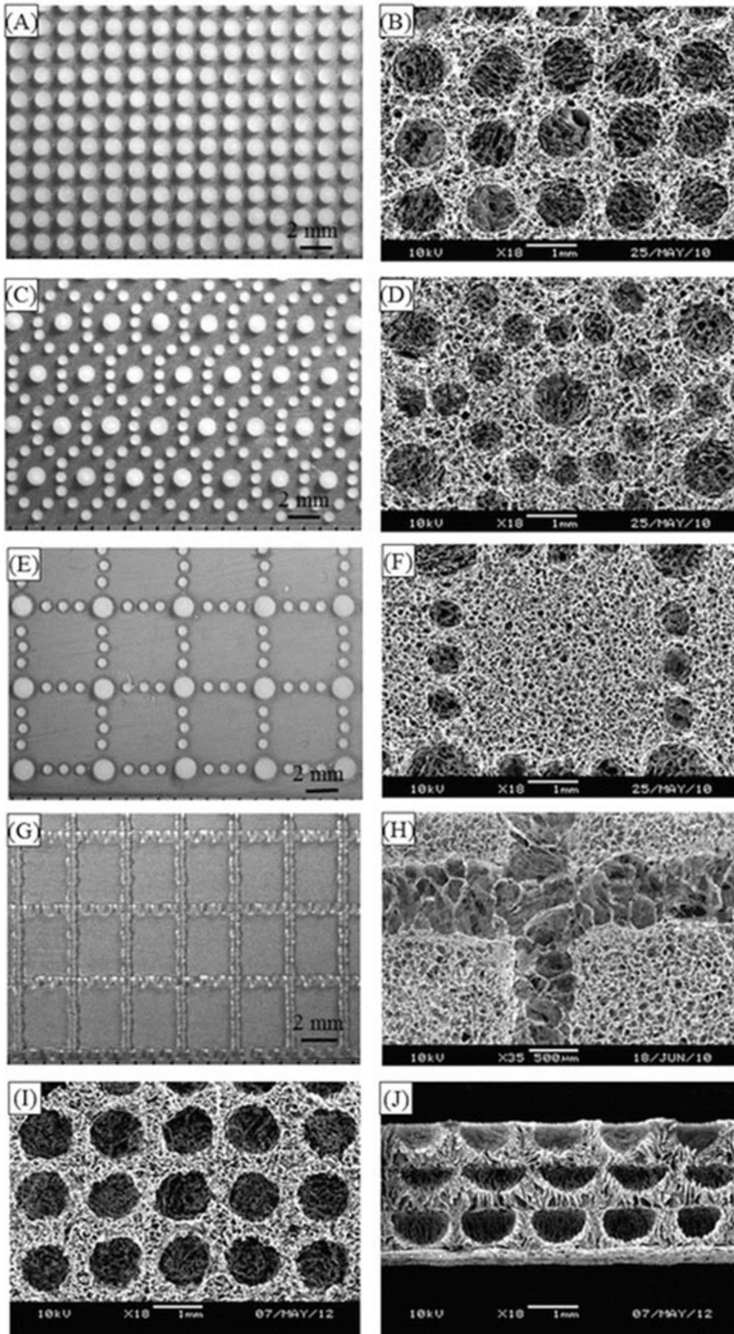

Fig. 2.2 Light microscopy micrographs of four types of ice micropattern templates (**a**, **c**, **e**, **g**) and SEM images of collagen porous scaffolds with one layer of micropatterned pores that are prepared with the respective ice micropattern templates (**b**, **d**, **f**, **h**), and a collagen sponge with three-

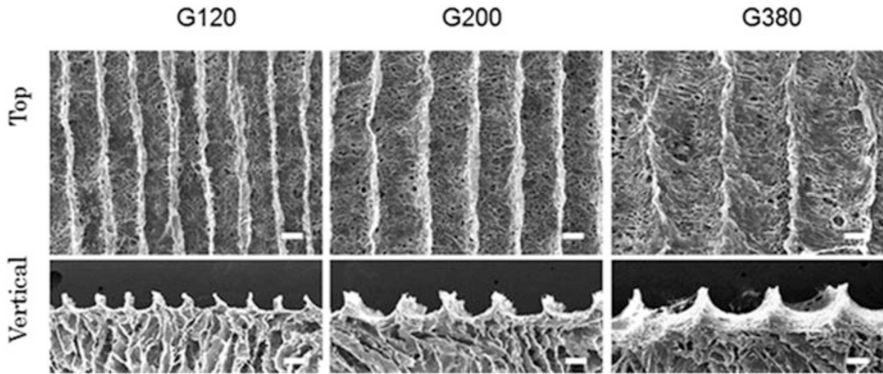

Fig. 2.3 SEM micrographs of microgroove collagen porous scaffolds with mean microgroove widths of 120, 200, and 380 μm. Upper images show the top view, and bottom images show the vertical cross-sectional view of the scaffolds. Scale bar = 100 μm. (Adapted from Ref. [33] with permission from Elsevier)

second layer of ice particulates and frozen. By repeating the procedure, polymer matrix embedded with multilayers of micropatterned ice particulates can be obtained. After freeze-drying, cross-linking, and washing, polymer porous scaffolds with 3D micropatterned pore structures are prepared. A collagen porous scaffold with 3D micropatterned pore structure is shown in Fig. 2.2i, j. The cross-section SEM image shows the stacked 3D pore structure.

Microgroove collagen porous scaffolds have been prepared with this method by using micropatterned ice lines as a template [33]. By controlling the width of ice lines, three types of collagen porous scaffolds with microgroove width of 120, 200 and 380 μm are prepared (Fig. 2.3). They are referred as G120, G200, and G380. The microgroove porous scaffolds have aligned concave microgrooves that exhibit semicircular shape in cross-sections.

The collagen microgroove porous scaffolds have been used for culture of L6 skeletal myoblasts for skeletal muscle tissue engineering. The myoblasts aggregate and form bundles in the microgroove scaffolds. The width of microgrooves has some effects on cell orientation and cell bundle formation. Scaffolds with wide microgrooves (G200 and G380) enable the formation of discrete cell bundles after 14 days of culture. Scaffolds with narrow microgrooves (G120) result in the formation of some cell bundles in microgrooves and mostly cellular flakes covering most of the area of scaffolds. Staining of myosin heavy chain (MHC) shows that well-aligned myotubes are formed in G200 and G380, while in G120 some myotubes are aligned in microgrooves and other myotubes in cellular flakes have random orientation.

Fig. 2.2 (continued) dimensional micropattern pores prepared with an ice micropattern template shown in **a** (**i** top surface, **j** cross-section). (Adapted from Ref. [32] with permission from John Wiley and Sons)

Furthermore, the embossing ice particulate method can be used to micropattern bioactive molecules in 3D porous polymer scaffolds. As an example, collagen porous scaffolds with micropatterned fibronectin, VEGF, and NGF have been prepared by the method [34, 35]. In this case, a collagen aqueous solution containing the bioactive molecules, other than pure water, is used to prepare the micropatterned ice lines. The ice micropatterns of the mixture of collagen/bioactive molecules are used to prepare collagen porous scaffolds having micropatterns of bioactive molecules. Not only single bioactive molecule, but also a few types of bioactive molecules can be co-micropatterned in the porous scaffolds. The bioactive molecules can be mixed and micropatterned together, or the bioactive molecules can be micropatterned separately to construct the porous scaffolds having co-micropatterns of a few types of bioactive molecules. Collagen porous scaffolds with micropatterned NGF and VEGF show stimulative effects on the regeneration of neural network and capillary network, respectively.

2.4 Scaffolds Prepared with Sacrificial Templates

Porous templating structures of biodegradable polymers have been used as sacrificial templates to generate interconnected pores in scaffolds [36–39]. PLGA sponges and PLGA meshes have been used as the sacrificial template because their degradation can be accelerated at a high pH. Unlike the ice particulates, PLGA sponges and PLGA meshes have integral, and continuous frame structures which negative replica form the interconnected pore structures in the scaffolds. Collagen scaffolds with interconnected pore structures prepared by this method are shown in Fig. 2.4 [36]. At first, PLGA sponge templates are prepared. Six types of PLGA sponges are prepared and used as the templates. A solvent casting/particulate leaching method is used to

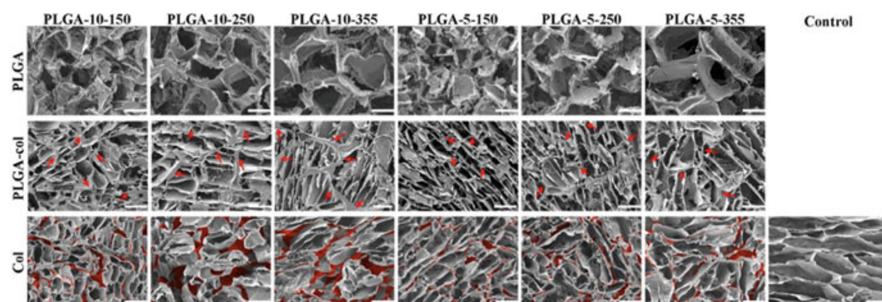

Fig. 2.4 SEM micrographs of the PLGA sponge templates, PLGA-collagen sponges (PLGA-col), and collagen sponges (Col). The central cross-sections of the sponges are used for SEM observation. The pore walls of the PLGA sponge templates are indicated by red arrows in the middle row micrographs (PLGA-col). The negative replica spaces of the PLGA sponge templates after their removal are indicated by the red marks in the bottom row micrographs (Col). Scale bar is 200 μm. (Adapted from Ref. [38] with permission from RSC)

the PLGA sponge templates by using NaCl particulates of three sizes (diameter of 150–250, 250–355, 355–500 μm). The ratio of PLGA/NaCl is 10:90 and 5:95. The PLGA sponge templates prepared with the different ratio of PLGA/NaCl and different size of NaCl particulates are designated as PLGA-10-150, PLGA-5-150, PLGA-10-250, PLGA-5-250, PLGA-10-355, and PLGA-5-355 (Fig. 2.4). The PLGA sponge templates have pore structures controlled by the NaCl particulate and PLGA/NaCl ratio. Their pore size is almost the same as that of the NaCl particulates. The PLGA-10-355 and PLGA-5-355 sponge templates prepared with 355–500 μm NaCl particulates have the largest pore size. Thickness of the pore walls increase with the PLGA/NaCl ratio.

And then, the PLGA sponge templates are immersed in 1 wt% collagen aqueous solution under vacuum to fill all the pores with collagen aqueous solution. After freeze-drying and crosslinking, PLGA-collagen sponges are prepared. 50 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 20 mM *N*-hydroxysuccinimide are used for the cross-linking, which is conducted in 95 (v/v)%, 90 (v/v)%, and 80 (v/v)% ethanol aqueous solutions, each for 3 h. SEM observation shows that hybridization with collagen results in the formation of collagen microsponges in the pores of the PLGA sponge templates (Fig. 2.4).

Finally, the PLGA sponge templates are selectively removed by accelerated degradation via immersion of the PLGA-collagen sponges in a 3 (wt/v)% ammonia hydroxide solution. The collagen component in the PLGA-collagen sponges remains intact during the accelerated degradation process. Collagen sponges with interconnected pore structures are formed after selectively removing the PLGA sponge templates. There are designated as Col-10-150, Col-5-150, Col-10-250, Col-5-250, Col-10-355, and Col-5-355. Collagen sponge prepared with direct freeze-drying of 1 wt% collagen aqueous solution without PLGA sponge templates is used as a comparison (control).

SEM observation shows the interconnected pore structures of the collagen scaffolds prepared with PLGA sponge templates (Fig. 2.4). The negative replica spaces of the PLGA sponge templates form the interconnecting channels among the pores of the collagen scaffolds. The character of the interconnecting channels is dependent on the frame structures of the PLGA sponge templates. The interconnecting channels become wider when the pore walls of the PLGA sponge templates are thick. The thickness of the interconnecting channels in the collagen scaffold prepared with PLGA-10-355 template was widest. The collagen sponges are used for culture of bovine articular chondrocytes and human bone marrow-derived mesenchymal stem cells (hMSCs). The cells can migrate into the pores through the interconnecting channels. The interconnecting channels in all the collagen sponges prepared with PLGA sponge templates facilitate cell migration and homogeneous distribution. Cell distribution in the collagen sponge prepared with PLGA-10-355 template is most homogeneous.

Besides the PLGA sponge templates, PLGA mesh has been used as sacrificial templates to fabricate mesh-like collagen scaffolds [39]. Mesh-like collagen scaffolds with large and small sizes are prepared by using PLGA mesh template (Fig. 2.5). The mesh-like collagen scaffold of large size is fabricated by forming a

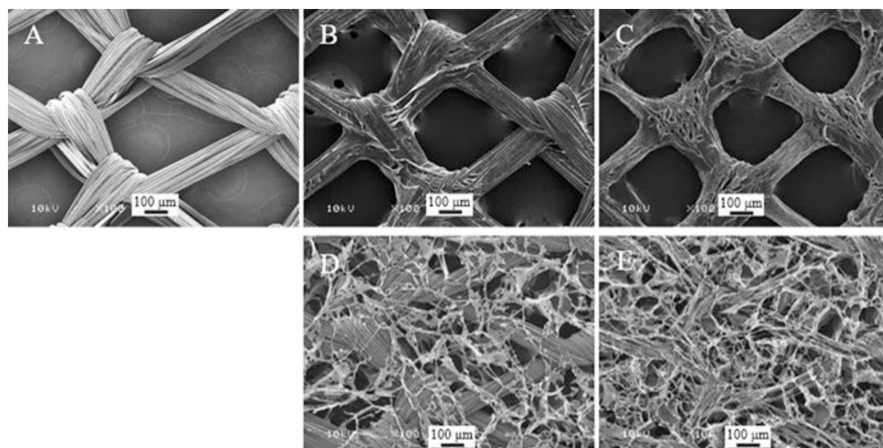

Fig. 2.5 SEM micrographs of PLGA mesh (**a**), collagen-coated PLGA mesh (**b**), mesh-like collagen scaffold of large size (**c**), PLGA-collagen composite mesh (**d**), and mesh-like collagen scaffold of small size (**e**). (Adapted from Ref. [36] with permission from RSC)

thin coating layer of collagen on the PLGA mesh template surface, followed by selective removal of the PLGA mesh template. Removal of PLGA mesh template is conducted by the accelerated degradation of PLGA, which is the same as above-mentioned. The mesh-like collagen scaffold of small size is fabricated by selective removal of PLGA mesh template from PLGA-collagen composite mesh which is fabricated by introducing collagen microsponges in the open spaces of the PLGA mesh template.

As shown in Fig. 2.5, the collagen-coated PLGA mesh has a similar pore structure to that of the PLGA mesh template. The mesh-like collagen scaffold of large size also has a similar pore structure. However, SEM observation of the cross-sections shows the cross-sections of the mesh-like collagen scaffold have microtubular structures. The microtubular structures are generated from the negative replica of PLGA fibers. The mesh-like collagen scaffold of small size has similar pore structure to that of the PLGA-collagen composite mesh, while its cross-sections have microtubular structures. Thickness of the mesh-like collagen scaffolds is controlled by the thickness of the PLGA mesh templates. The mesh-like collagen scaffolds can be used for tissue engineering of thin tissues. When human dermal fibroblasts are cultured in the mesh-like collagen scaffolds, they support cell adhesion and promote cell proliferation. The fibroblasts form layered structures more rapidly in the mesh-like collagen scaffold of small size than in the mesh-like collagen scaffold of large size.

The sacrificial PLGA meshes have also been used to prepare extracellular matrices (ECM) scaffolds [40, 41]. At first, cells are cultured in the PLGA meshes. The cells proliferate and excrete their own extracellular matrices. Subsequently, the cellular components are removed by decellularization after the cells have excreted enough amount of extracellular matrices. Finally, the templates are selectively

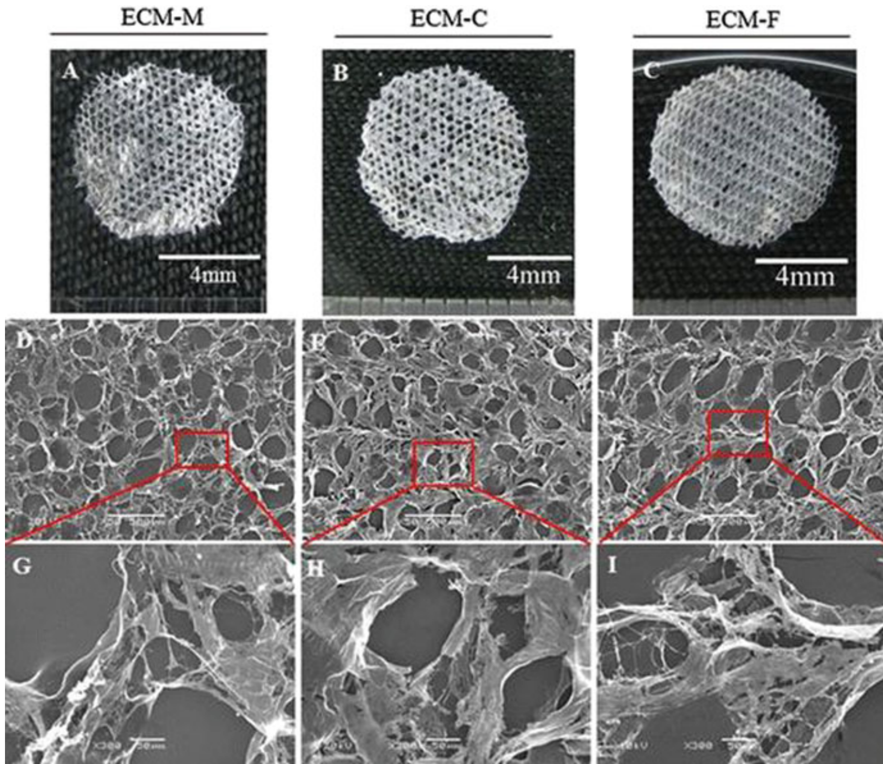

Fig. 2.6 Gross appearance (**a**–**c**) and SEM micrographs (**d**–**i**) of ECM porous scaffolds prepared from hMSCs (**a**, **d**, **g**), chondrocytes (**b**, **e**, **h**), and fibroblasts (**c**, **f**, **i**). Scale bar = 500 μm in (**d**–**f**), and 50 μm in (**g**–**i**). (Adapted from Ref. [40] with permission from Elsevier)

removed, while the extracellular matrices are remained. After cross-linking, ECM scaffolds are obtained. ECM scaffolds have been prepared from human bone marrow mesenchymal stem cells (hMSCs), human articular chondrocytes, and human dermal fibroblasts by the method. The ECM scaffolds from hMSCs (ECM-M), chondrocytes (ECM-C), and fibroblasts (ECM-F) have a mesh-like appearance similar to that of the PLGA mesh template (Fig. 2.6). The ECM scaffolds have different composition that is dependent on the cell type and culture condition.

2.5 Composite Porous Scaffolds

Porous scaffolds of biodegradable synthetic polymers, and naturally derived polymers have their respective advantages and problems. Porous scaffolds prepared from synthetic biodegradable polymers such as PGA, PLA, PLGA, and PCL have relatively strong mechanical strength. Their degradation can be controlled by

crystallinity, molecular weight, and copolymer ratio of the polymers. However, synthetic polymer scaffolds are devoid of cell recognition signals, and their hydrophobic surface property hinders smooth cell seeding. On the other hand, naturally derived polymers, such as collagen, gelatin, and hyaluronic acid, have the advantages of specific cell interactions and hydrophilicity, while their mechanical property is inferior to synthetic polymer scaffolds. Biodegradable synthetic polymers, and naturally derived polymers have hybridized to prepare their composite scaffolds to combine the advantageous properties of both types of polymers, and overcome their drawbacks [25, 42, 43]. One type of hybridization is to form microsponges of naturally derived polymers in the void spaces or opening of a porous skeleton of biodegradable synthetic polymers [44–47]. The void space or opening of biodegradable synthetic polymer porous skeleton is filled with microsponges of naturally derived polymers. The pore surface of biodegradable polymer porous skeleton is also coated with naturally derived polymers. When the composite scaffolds are used for 3D cell culture, cells only contact and interact with naturally derived polymers. The porous skeleton of biodegradable polymers serves as a mechanical skeleton to provide necessary mechanical strength to support the whole scaffolds. Another type of hybridization is to construct naturally derived polymer porous structures in the open space of a cup, cage, or cylinder of biodegradable synthetic polymers [48, 49]. All the composite porous scaffolds have high mechanical strength, good cell interaction and surface hydrophilicity.

As a typical example of composite scaffolds, PLGA-collagen composite mesh can be prepared by introducing collagen microsponges in the interstices of a PLGA knitted mesh [46, 47]. Collagen sponge can also be formed on one side of the PLGA knitted mesh or both sides of the PLGA knitted mesh to construct semi-type or sandwich-type PLGA-collagen composite scaffolds [50]. The semi-type and sandwich-type PLGA-collagen composite scaffolds have been used for culture of bovine articular chondrocytes for cartilage tissue engineering. Both composite scaffolds show a spatially even cell distribution, natural chondrocyte morphology, abundant cartilaginous extracellular matrix deposition, and excellent biodegradation in vivo. The histological structure and mechanical properties of the engineered cartilage using the semi-type and sandwich-type composite scaffolds match the native bovine articular cartilage. The composite scaffolds are useful for tissue engineering and regenerative medicine.

2.6 Biomimetic ECM Scaffolds

ECM are a complex network composed of a variety of proteins and proteoglycans. ECM play a very important role in regulation of cell functions. ECM derived from decellularized tissues have been widely explored as a source of biological scaffolds for tissue engineering. Acellular ECM has been prepared by decellularization of tissues and organs, such as the small intestinal submucosa, heart valve, blood vessel, skin, nerve, tendon, ligament, urinary bladder, vocal fold, amniotic membrane, heart,

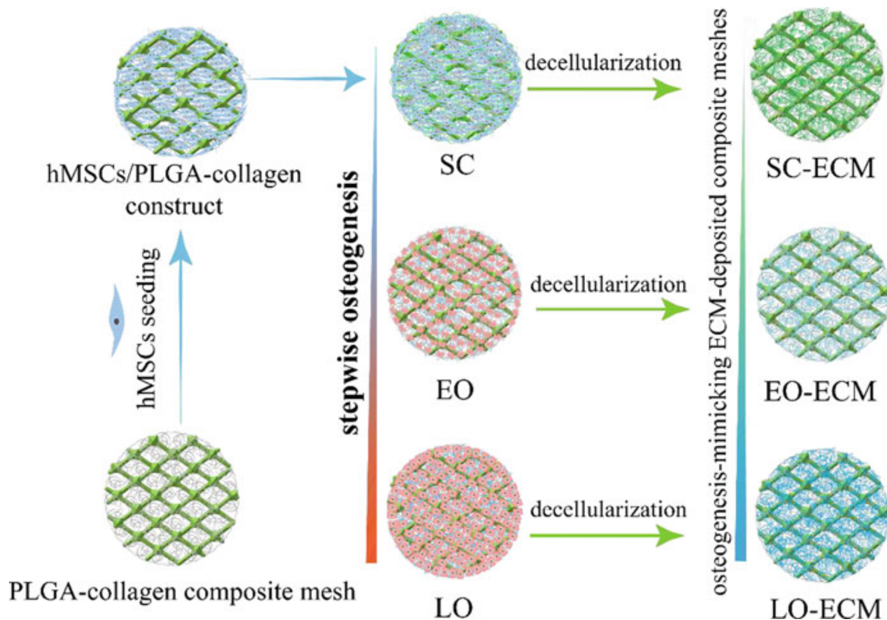

Fig. 2.7 Fabrication scheme of PLGA-collagen-ECM composite meshes mimicking the ECM composition of stepwise osteogenesis. (Adapted from Ref. [64] with permission from IOP Science)

liver, and lung [4]. The ECM scaffolds obtained from decellularized tissues and organs offer the advantage of maintaining the structures of the respective tissues and organs. However, they suffer from problems of autologous tissue/organ scarcity, host responses, and pathogen transfer when allogeneic and xenogeneic tissues and organs are used.

Cell culture method has been adopted as an alternate method to prepare the ECM scaffolds [51–58]. Cell-derived ECM have been used to fabricate various scaffolds for tissue engineering applications [59, 60]. Cultured cells offer several advantages of pathogen-free and availability over the decellularization of tissues and organs. The method can be adopted to fabricate ECM scaffolds mimicking the dynamically remodeled ECM compositions. Differentiation of stem cells to mature cells has been reported to pass through stepwise stages of maturation [61, 62]. ECM are dynamically changed and remodeled during the stepwise development process [63]. By controlling the stepwise differentiation of stem cells into different lineage, ECM scaffolds mimicking stepwise osteogenesis, stepwise adipogenesis, and stepwise chondrogenesis are fabricated.

As shown in Fig. 2.7, PLGA-collagen-ECM composite meshes that mimic the dynamically remodeled ECM composition of stepwise osteogenesis have been fabricated by controlling the differentiation stages during osteogenic differentiation of hMSCs in the PLGA-collagen composite meshes [64]. When hMSCs are cultured in the PLGA-collagen composite meshes, hMSCs secret their ECM that are

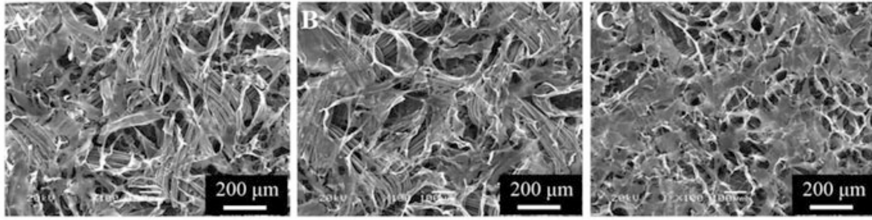

Fig. 2.8 SEM micrographs of PLGA-collagen-ECM scaffolds mimicking stem cell ECM (**a**), early osteogenesis stage ECM (**b**), and late osteogenesis stage ECM (**c**). (Adapted from Ref. [64] with permission from IOP Science)

deposited in the composite meshes. The osteogenic differentiation stages can be controlled at stem cell stage, early osteogenesis stage, and late osteogenesis stage by using proliferation medium or osteogenic differentiation medium, and by adjusting culture time. The stem cell stage ECM (SC-ECM) are deposited in the composite meshes by culturing hMSCs in basal medium for 7 days. The early osteogenesis stage ECM (EO-ECM) are deposited in the composite meshes by culturing hMSCs in osteogenic medium for 7 days. During 7 days culture in osteogenic differentiation medium, the cells express high level of early-stage osteogenesis marker, alkaline phosphatase (ALP), while no calcium deposition which is a late-stage marker of osteogenesis is detected. The late osteogenesis stage ECM (LO-ECM) are deposited in the composite meshes by culturing hMSCs in osteogenic medium for 21 days. The late osteogenesis stage is confirmed by high expression of ALP and calcium deposition.

After decellularization of the hMSCs/scaffold constructs, the three types of stepwise ECM scaffolds are obtained. The porous structures of the stepwise osteogenesis-mimicking ECM scaffolds are shown in Fig. 2.8. The ECM scaffolds have different compositions which are dependent on the osteogenic differentiation stage of hMSCs.

The stepwise osteogenesis-mimicking ECM scaffolds show different osteogenesis-induction effects on hMSCs. The EO-ECM scaffold shows a promotive effect on osteogenic differentiation of hMSCs, and the LO-ECM scaffold has a moderate effect on osteogenic differentiation of hMSCs. However, the SC-ECM scaffold exhibits an inhibitory effect on osteogenic differentiation of hMSCs. The varied effects of the ECM scaffold on osteogenic differentiation of hMSCs are related with the dynamically remodeling ECM components in the stepwise osteogenesis-mimicking ECM scaffolds.

By the same method, stepwise adipogenesis-mimicking ECM-deposited PLGA-collagen composite meshes have been prepared by controlling the adipogenic differentiation stages of hMSCs in the PLGA-collagen composite meshes [65]. The ECM components are dependent on the adipogenic differentiation stages. The stepwise adipogenesis-mimicking ECM scaffolds show different effects on adipogenic differentiation of hMSCs. The ECM scaffold fabricated from early stage of adipogenesis enhances the adipogenic differentiation, while the ECM

scaffold prepared from stem cell stage or late stage of adipogenesis show an inhibitive effect on adipogenic differentiation of hMSCs.

The method has also been used to prepare ECM scaffolds mimicking endochondral ossification-related ECM by depositing ECM secreted from stem cells (SC-ECM), chondrogenic (CH-ECM), hypertrophic (HY-ECM) and osteogenic (OS-ECM) stages of hMSCs in the PLGA-collagen composite mesh [66]. During bone tissue development, endochondral ossification (ECO) occurs. ECM play an important role in ECO. The SC-ECM, CH-ECM, HY-ECM and OS-ECM are adopted for culture of hMSCs to elucidate the effects of biomimetic ECM mimicking the ECO-related ECM on differentiation of hMSCs. Their effects on osteogenic differentiation of hMSCs are different. Their promotive effect on osteogenic differentiation of hMSCs is an order of HY-ECM scaffold > CH-ECM scaffold, OS-ECM scaffold > SC-ECM scaffold. Their effects on chondrogenic or adipogenic differentiation are almost the same. Therefore, the HY-ECM may be important for ECO.

Furthermore, ECM scaffolds mimicking the dynamically remodeling ECM during simultaneous osteogenic and adipogenic differentiation of hMSCs have been fabricated by simultaneously controlling osteogenic and adipogenic differentiation of hMSCs in the PLGA-collagen composite meshes [67]. The simultaneous osteogenic and adipogenic differentiation of hMSCs is controlled at four stages, early osteogenesis/early adipogenesis (EOEA-ECM), early osteogenesis/late adipogenesis (EOLA-ECM), late osteogenesis/early adipogenesis (LOEA-ECM), and late osteogenesis/late adipogenesis (LOLA-ECM). Mixtures of osteogenic induction medium and adipogenic induction medium at different ratios are adopted to control the simultaneous osteogenic and adipogenic differentiation. The compositions of the ECM scaffolds vary according the different stages of simultaneous differentiation stages. They also show different effects on adipogenic and osteogenic differentiation of hMSCs. The EOEA-ECM scaffold has a promotive effect on adipogenesis, while a suppressive effect on osteogenesis. The LOEA-ECM and LOLA-ECM scaffolds show a promotive effect on osteogenesis and a moderate effect on adipogenesis. The EOLA-ECM scaffold exhibits a suppressive effect on both osteogenesis and adipogenesis. The varied effects of the ECM scaffolds on hMSCs differentiation are dependent on their ECM compositions. These ECM scaffolds can be used as models for 3D cell culture for investigation of ECM-cell interaction and tissue engineering applications.

2.7 Summary

Porous scaffolds can be prepared from a number of biodegradable polymers and extracellular matrices. Their composition can be controlled by combining a few types of polymers and matrices. Biodegradable synthetic polymers and naturally derived polymers can also be hybridized to overcome the drawbacks of single polymers. Their porous structures can be controlled by using different fabrication methods. Free ice particulate method, embossing ice particulate method, and

sacrificial template method can well control the porous structures and introduce micropatterns in the scaffolds. Cell-derived ECM scaffolds not only mimic the cellular microenvironment, but also mimic the dynamics of ECM remolding during stem cell differentiation or tissue development. The polymer porous scaffolds and biomimetic ECM scaffolds can be widely used for engineering of various tissues and organs.

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