Design of artificial extracellular matrices for tissue engineering

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A B S T R A C T

The design of artificial extracellular matrix (ECM) is important in tissue engineering because artificial ECM regulates cellular behaviors, including proliferation, survival, migration, and differentiation. Artificial ECMs have several functions in tissue engineering, including provision of cell-adhesive substrate, control of three-dimensional tissue structure, and presentation of growth factors, cell-adhesion signals, and mechanical signals. Design criteria for artificial ECMs vary considerably depending on the type of the engineered tissue. This article reviews the materials and methods that have been used in fabrication of artificial ECMs for engineering of specific tissues, including liver, cartilage, bone, and skin. This article also reviews artificial ECMs used for modulation of stem cell behaviors for tissue engineering applications.

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A R T I C L E   I N F O

Article history:
Received 25 May 2010
Received in revised form 22 September 2010
Accepted 7 October 2010
Available online 20 October 2010

Keywords:
Artificial extracellular matrix
Naturally-derived polymer
Stem cells
Synthetic polymer
Tissue engineering

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0079-6700/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.progpolymsci.2010.10.001
Tissue engineering, which aims to reconstruct living tissues for replacement of damaged or lost tissues/organs of living organisms, has recently emerged as an exciting interdisciplinary area in the life sciences [1]. To achieve this aim, it is necessary to use cells together with biosignalling molecules and extracellular matrix (ECM) into or onto which cells will develop, organize, and behave as if they are in their native tissue [2]. Tissue engineering typically uses artificial ECMS to engineer new tissues from cells [3,4]. Artificial ECMS should be designed to bring the desired cell types in contact with an appropriate environment and to provide mechanical support until the newly formed tissues are structurally stable [5]. Therefore, design of artificial ECMS is very important in tissue engineering, because artificial ECMS regulate many cellular behaviors, including proliferation and growth, survival, change of cell shape, migration, and differentiation [6]. Also, artificial ECMS have multiple functions, such as serving as an adhesive substrate, provision of structure, presentation and storage of growth factors, and detection of signals [7].
Table 1
General characteristics of commonly used naturally-derived polymers for tissue engineering [modified from Ref. [9]].

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Biocompatibility</th>
<th>Disadvantages</th>
<th>Biodegradability</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Minimal cytotoxicity,</td>
<td>Proteolytic removal of small nonhelical telopeptides</td>
<td>Bulk, Controllable</td>
<td>Skin; cartilage; bone; ligaments; tendons; vessels; nerves; bladder; liver</td>
</tr>
<tr>
<td></td>
<td>Mild foreign body reaction,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimal inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>Minimal foreign body reaction,</td>
<td>Highly viscous solution, Many purification steps</td>
<td>Bulk, 1 h to 1 month</td>
<td>Skin; cartilage; bone; ligaments; nerves; vessels; liver</td>
</tr>
<tr>
<td></td>
<td>No inflammation</td>
<td>after chemical modification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alginic acid</td>
<td>Minimal foreign body reaction,</td>
<td>Uncontrollable dissolution of hydrogel</td>
<td>Bulk, 1 day to 3 months</td>
<td>Skin; cartilage; bone; nerves; muscle; pancreas</td>
</tr>
<tr>
<td></td>
<td>No inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitosan</td>
<td>Minimal foreign body reaction,</td>
<td>Uncontrollable deacetylation and molecular weight</td>
<td>Bulk, 3 days to 6 months</td>
<td>Skin; cartilage; bone; nerves; vessels; liver; pancreas</td>
</tr>
<tr>
<td></td>
<td>No inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>Minimal cytotoxicity,</td>
<td>Weak mechanical property</td>
<td>Bulk, Controllable</td>
<td>Skin; bone; cartilage; ligaments; breast</td>
</tr>
<tr>
<td></td>
<td>Mild foreign body reaction,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimal inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrin</td>
<td>Minimal cytotoxicity,</td>
<td>Weak mechanical property</td>
<td>Bulk, Controllable</td>
<td>Skin, bone, cartilage; liver; tendons; ligaments; vessels</td>
</tr>
<tr>
<td></td>
<td>Mild foreign body reaction,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimal inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(hydroxyalkanoate)</td>
<td>Minimal cytotoxicity,</td>
<td>Pyrogen removed</td>
<td>Bulk, Controllable</td>
<td>Skin; bone; tendons; cartilage; nerves; ligaments; heart; vessels; muscle</td>
</tr>
<tr>
<td></td>
<td>Mild foreign body reaction,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimal inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silk</td>
<td>Minimal cytotoxicity,</td>
<td>Inflammation of sericin</td>
<td>Bulk, Controllable</td>
<td>Skin; ligaments; bone; cartilage; tympanic membrane; vessels; tendons</td>
</tr>
<tr>
<td></td>
<td>Mild foreign body reaction,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimal inflammation</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Clinical trials. Naturally-derived polymers have advantages over synthetic ones because they have excellent biological properties, including cell adhesion, mechanical properties similar to those of natural tissues, biodegradability, and biocompatibility [12]. General characteristics of the most commonly used naturally-derived polymers for tissue engineering are shown in Table 1. A recent review on tissue engineering using natural polymer-based artificial ECMs is available [10].

Synthetic polymer-based artificial ECMs are generally fabricated from biocompatible, biodegradable polymers to avoid chronic foreign body reactions. Synthetic polymers may be obtained by reproduction on a large scale and can be processed into artificial ECMs in which the mechanical properties and degradation time of some synthetic polymers can be controlled [12]. However, the greatest disadvantage of synthetic polymers is the lack of cell-recognition sites and the possibility of a pro-inflammatory response after implantation. General characteristics of commonly used synthetic polymers for tissue engineering are shown in Table 2. Recent reviews on synthetic polymers for tissue engineering scaffold are available [9,11,12].

2. Liver

The liver plays an important role in a broad spectrum of physiological functions, including metabolism, storage, and synthesis and release of vitamins, carbohydrates, proteins, lipids, and cyclic tetrapyrroles [13]. Also, the liver detoxifies and inactivates endogenous and exogenous substances, and activates precursor molecules [13]. Therefore, the design of artificial ECMs for liver tissue engineering is critically important, because hepatocytes are attachment-dependent cells and lose their liver-specific functions without an optimal ECM. This section discusses mechanisms of the specific interaction between hepatocyte and galactose moieties, classification of artificial ECMs, factors affecting cellular behaviors of hepatocytes, and surface modification.

2.1. Mechanism of specific interaction between hepatocytes and galactose moieties

Asialoglycoprotein receptors (ASGPR) of hepatocytes were first identified by Pricer et al. [14]; circulating asialoglycoproteins (ASGPs) bind to and are degraded by hepatocytes. Hepatocytes have cell surface receptors that recognize and bind molecules with exposed galactose, N-acetyl galactosamine, or glucose residues [15]. After binding to receptors, ASGPs are internalized via coated pits and coated vesicles and subsequently appear in a complex arrangement of larger smooth-surface vesicles [16]. Hepatic plasma membrane receptors localized on the sinusoidal face of hepatocytes mediate specific binding and uptake of partially deglycosylated glycoproteins through receptor-mediated endocytosis [17]. Therefore, mimicking the biological environment, the process of incorporation of old proteins by the liver has been imitated; however, hepatocyte transplantation using poly(lactic acid) (PLA) [18], poly(vinyl alcohol) (PVA) [19], poly(glycolic acid) (PGA) [20], poly(lactic-co-glycolic acid) (PLGA) [21], collagen [22], alginate [23], and polyurethane [24] has been achieved in vivo.
2.2. Classification of artificial ECMs for liver tissue engineering

Two types of artificial ECMs for liver tissue engineering will be discussed: one is derived from synthetic polymers, and the other is derived from natural sources.

2.2.1. Synthetic polymers

Synthetic polymer applications in liver tissue engineering have attracted attention in recent years due to their excellent mechanical properties, processability, low cost, and controllable degradation time. However, the greatest disadvantage of synthetic polymers is the absence of hepatocyte-recognition sites. Therefore, many efforts have focused on incorporation of galactose ligands, the cell-adhesion sites for synthetic polymers, into the design of biomimetic polymers for hepatocytes.

Galactose ligand was first coupled to polyacrylamide (PAAm) by Weigel et al. [25]. PAAm gels were synthesized by copolymerization of N-succinimidyl acrylate, (PAAm) by Weigel et al. [25]. PAAm gels were synthesized by copolymerization of N-succinimidyl acrylate, polyacrylamide in a Ca²⁺- and temperature-dependent manner via a patch of ASGPR in rat hepatocytes, but not chicken hepatocytes [25,27]. Also, cell binding to the polymer was inhibited by asialo-orosomucoid, indicating a receptor-mediated mechanism [28]; however, the effect of galactose-coupled PAAm gel hydration on hepatocyte adhesion was not investigated.

Akaike and colleagues synthesized galactose-carrying polystyrene (PS) in order to guide hepatocyte adhesion. Galactose-carrying PS [poly(N-p-vinylbenzyl-4-O-β-d-galactopyranosyl-d-glucosamide), PVLA] was synthesized according to three steps, as shown in Fig. 1 [29]. Synthesis of PVLA is relatively simple, protection of the hydroxyl groups of lactose is unnecessary, and the yield of each step is high, even though it is expensive to prepare compared to other synthetic polymers. The authors investigated the use of PVLA as an artificial ECM for hepatocytes. The results indicated that hepatocytes adhered to the PVLA through a unique ASGPR-galactose interaction between the ASGPR of hepatocytes and highly concentrated galactose moieties along the polymer chains [30]. The round morphologies of hepatocytes on PVLA that adhered at high concentrations of PVLA triggered spheroid formation of hepatocytes in the presence of epidermal growth factor (EGF), leading to enhanced differentiation functions [31]. In addition, due to its amphiphilic character, this polymer is stably adsorbed onto the tissue culture dish [32].

Lopina et al. synthesized galactose-modified star poly(ethylene oxide) (PEO) hydrogels by reacting trehalose-4,6-diols and glycerol-β-d-galactopyranosyl-β-d-glucosamine, (PEO-galactose) hydrogels with 1-amino-1-deoxy-β-d-galactose [33]. They investigated hepatocyte attachment on these constructs. Hepatocytes were found to exhibit galactose-specific adhesion to the galactose-modified PEO gels, adhering to gels with galactose via the spreading morphologies of hepatocytes at low concentrations of immobilized ligands, but not glucose [34].

Donati et al. synthesized galactosylated poly(styrene-co-maleic acid) (PSMA) by combining PSMA with 1-amino-1-deoxy-β-d-galactose (or 1-amino-1-deoxy-β-d-lactose)}
and testing HepG2 adhesion [35]. The results showed that cell adhesion on galactosylated PSMA or lactosylated PSMA was approximately five times higher than that of PSMA itself.

Hepatocyte–ECM interaction is a surface phenomenon, and is affected by surface properties of ECM; however, bulk properties of the ECM dictate the mechanical properties of the ECM. Therefore, surface modification of the ECM with galactose ligand is often necessary for optimization of ECM for hepatic tissue engineering. Yoon et al. synthesized galactosylated PLGA film or galactosylated PLGA sponge by reacting the carboxylic acid of PLGA at the terminal group and N-(amino butyl)-O-β-D-galactopyranosyl-(1→4)-D-gluconamide or by reacting the amino groups of PLGA-PEG and lactobionic acid for hepatocyte culture [36], even though the albumin secretion rates of the galactosylated PLGA surface in vitro were relatively low compared to those of other culture systems. However, using the perfusion culture system based on galactose-modified macroporous PLGA scaffolds under optimal flow conditions, the rate of albumin secretion was significantly increased due to spheroid formation of hepatocytes in the scaffolds [36].

Mao and colleagues immobilized galactose ligands on acrylic acid-graft-copolymerized poly(ethylene terephthalate) (PET) film by plasma treatment and evaluated hepatocyte function [37]. The results indicated that hepatocytes cultured on the galactosylated surface exhibited good albumin and urea synthesis, which were comparable to those of hepatocytes cultured on the collagen-modified PET surface. Also, they prepared the galactosylated PET surface by surface-grafting poly(acrylic acid) on plasma-pretreated PET film to increase the density of the immobilized galactose ligands [37]. The results indicated that due to high ligand density, better maintenance of albumin secretion and urea synthesis function was obtained compared to culture on a collagen-coated surface. Further, they prepared galactosylated poly(vinylidene difluoride) (PVDF) by coating with galactose-tethered Pluronic and evaluated hepatocyte function [38]. Results demonstrated that hepatocytes attached to galactosylated PVDF formed multi-cellular spheroids after one day of culture and exhibited higher albumin and P4501A1 detoxification than the unmodified PVDF membrane and collagen-coated surface. However, due to only hydrophobic interaction in the adsorption of galactose ligands, the stability of the coating after long culture periods is a substantial concern. Kang et al. prepared galactose-grafted PS surfaces by grafting of N-p-vinylbenzyl-4-O-β-D-galactopyranosyl-D-gluconamide to a PS dish using oxygen plasma glow discharge treatment and evaluated hepatocyte adhesion [39]. The results indicated that compared to PVLA, hepatocytes adhered more slowly to the galactose-grafted PS surface than to PVLA during the first 2 h of incubation due to the low galactose density of the galactose-grafted PS.

Higashiyama et al. prepared fructose- and galactose-modified polyamidoamine dendrimers by imine reaction between polyamidoamine dendrimer and galactose (or fructose) and evaluated hepatocyte function [40]. The results indicated that simultaneous modification of the dendrimer with fructose and galactose had a synergistic effect on spheroid formation of hepatocytes, higher urea synthesis, and increased albumin gene expression than those cultured on single-ligand modified dendrimers; the ligands introduced in the dendrimer were not verified. Synthetic polymers derivatized with galactose ligands are summarized in Table 3.

2.2.2. Naturally-derived polymers

Naturally-derived polymers composed of proteins, polysaccharides, and nucleic acids have several inherent merits, including bioactivity, the ability to present receptor-binding ligands to cells, biodegradability, and susceptibility to natural remodeling, even though antigenicity, instability/deterioration, complexity of purification, and risk of disease transmission should be considered [41]. This section discusses the liver tissue engineering applica-
hybrids were prepared as artificial ECMs for liver gen gels, collagen/Poloxamine and collagen/chitosan. To overcome the weak mechanical property of the collagen containing blood vessels, suggesting that this is an efficient method for engineering of hepatic tissue with formation of a large fused hepatic system containing blood vessels, suggesting that this is an efficient method for engineering of hepatic tissue in vivo. To overcome the weak mechanical property of the collagen gels, collagen/Poloxamine and collagen/chitosan hybrids were prepared as artificial ECMs for liver. Table 3

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Type of galactose</th>
<th>Linkage</th>
<th>Characters</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(acryl amide)</td>
<td>6-aminohexyl β-D-galactose</td>
<td>Amide</td>
<td>Firstly coupled temperature dependence of endocytosis, No results of effect of hydration on cell adhesion</td>
<td>[25–28]</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>β-n-lactose</td>
<td>Amide</td>
<td>Amphiphilic and micellar, Spheroid formation of hepatocytes, Non-degradable</td>
<td>[29–31]</td>
</tr>
<tr>
<td>Poly(ethylene oxide)</td>
<td>1-amino-1-deoxy-β-D-galactose</td>
<td>SN1 reaction</td>
<td>Radiation-crosslinked PEO star hydrogel spreading of hepatocytes, Spatial distribution of galactose ligand bands, No mechanism of cell adhesion</td>
<td>[33–34]</td>
</tr>
<tr>
<td>Poly(styrene-co-maleic acid)</td>
<td>1-amino-1-deoxy-β-D-galactose</td>
<td>Amide</td>
<td>Appearance of circular dichroism</td>
<td>[35]</td>
</tr>
<tr>
<td>Poly(lactic acid-co-glycolic acid)</td>
<td>N-(aminobutyl)-O-β-D-galactopyranosyl(1→4)-D-glucoamide Lactobionic acid</td>
<td>Amide</td>
<td>Introduction of PEG spacers, Spheroid formation of hepatocytes, Perfusion culture system</td>
<td>[36]</td>
</tr>
<tr>
<td>Acrylic acid-graft-poly(ethylene terephthalate)</td>
<td>1-O-(6-aminohexyl)-D-galactopyranoside</td>
<td>Amide</td>
<td>UV-induced graft copolymerization, Spheroid formation of hepatocytes, High albumin secretion and urea synthesis</td>
<td>[37]</td>
</tr>
<tr>
<td>Poly(vinylidene difluoride)</td>
<td>β-n-lactose</td>
<td>Imine reaction</td>
<td>Unstable coating due to physical adsorption, Buffering of proteins by PEO synthesis</td>
<td>[38]</td>
</tr>
<tr>
<td>Polysyrene</td>
<td>Galactose+fructose</td>
<td>Ether</td>
<td>No results of dependence of endocytosis, No results of cell adhesion</td>
<td>[39]</td>
</tr>
<tr>
<td>Polymidoamine dendrimer</td>
<td>Galactose+fructose</td>
<td>Imine reaction</td>
<td>Uncertain chemistry, Synergistic effect by fructose</td>
<td>[40]</td>
</tr>
</tbody>
</table>

Collagen is a family of multifunctional ECM known to play key roles in cell attachment and migratory cellular behaviors, such as embryogenesis, malignancy, homeostasis, wounding healing, and maintenance of tissue integrity [46]. The ability of fibronectin to serve as a substrate for cell adhesion is based on the RGD tripeptide [47]. However, due to the high cost, research on the use of fibronectin in tissue engineering, except for the coating of fibronectin on PS [48] and PLGA surfaces [49], has been limited. However, significant research has been conducted on the role of fibronectin in regulation of switching from differentiation to growth [50], tubulin monomer growth [51], cell cycle status [52], cell spreading [53], and motility [54]. The effect of hepatocyte growth factor (HGF) on hepatocytes was studied for elucidation of its basic molecular biology. Bhadriraju et al. [48] compared hepatocyte adhesion, growth, and differentiated function on 2.3 kD and 73 kD RGD peptide-coated PS dishes to that on fibronectin-coated ones. Of particular interest, a 2.3 kD RGD peptide induced a round cell shape, enhanced differentiated function, and inhibited DNA synthesis; however, the 73 kD RGD peptide induced cell spreading, dedifferentiation, and enhanced DNA synthesis (similar to the impact of fibronectin), suggesting that RGD peptide conformation determines the specificity of cellular response. Fiegel et al. [49] investigated the effect of three-dimensional culture
of rat hepatocytes on fibronectin-coated PLGA scaffolds under flow cell conditions. The results indicated that cellular functions of hepatocytes, such as growth and albumin secretion, were increased significantly by fibronectin coating and flow conditions.

Alginate, the primary structural component of brown seaweed, is the monovalent form of alginic acid, and is a linear polymer of β(1 → 4)-linked d-mannuronic acid and (1 → 4)-linked L-guluronic acid [56]. Alginate is widely used because it has low toxicity, is easy to modify chemically, and undergoes mild ionotropic gelation in the presence of divalent cations; however, degradation of alginate hydrogel occurs via a slow and unpredictable dissolution process in vivo [57]. Yang et al. prepared galactosylated alginate via the reaction of carboxylic acids of alginate and amine-modified lactobionic acid (Fig. 2) and evaluated the liver function of hepatocytes in galactosylated alginate microcapsules. The results indicated that binding of galactosylated alginate microcapsules with hepatocytes occurred via the ASGPR patch on hepatocytes and that liver function of hepatocytes was enhanced compared to that in alginate treated hepatocytes due to spheroid formation of hepatocytes in galactosylated alginate microcapsules.

Chitosan is a linear polymer of β(1 → 4)-linked d-glucosamine, and is obtained by deacetylation of chitin; it is suitable as a substrate for biomimetic glycopolymers due to the similarity of its structure to glycosaminoglycans found in native tissue [57]; however, it is only soluble in diluted acids. Free amino groups in chitosan are easily modified by covalent attachment of molecules using carbodiimide chemistry. Park et al. prepared galactosylated chitosan (GC) by reacting lactobionic acid with amino groups of chitosan using EDC/NHS as coupling agents (Fig. 3) and evaluated the subsequent hepatocyte adhesion [59]. The results indicated that hepatocytes adhered via galactose-specific recognition between GC and ASGPR of hepatocytes, and hepatocytes that adhered to the surface at high concentrations of GC exhibited substantial spheroid formation after 24 h exposure to EGF. Yang et al. also prepared a hybrid artificial ECM composed of alginate and GC because the mechanical properties of GC were insufficient [60,61]. Hybrid GC and alginate were found to improve cell adhesion and stability of the artificial ECM, which retained differentiated cellular functions better than alginate itself. Also, pore size of the artificial ECM could be controlled by the content and molecular weight of GC and the freezing temperature of alginate/GC hydrogel. Introduction of heparin in the alginate/GC artificial ECM enhanced the level of albumin secretion from hepatocytes in the presence of HGF due to rapid formation of stable spheroids in the alginate/GC/heparin artificial ECM, which is closely related to connexin 32 and E-cadherin genes in cell-to-cell interactions [62].

Gelatin is a naturally-derived polymer obtained from collagen and has been used in medical and pharmaceutical applications because of its biodegradability and biocompatibility [63]. Hong et al. prepared galactosylated gelatin
by reacting lactobionic acid with amine-incorporated gelatin (Fig. 4) and evaluated it for use in hepatocyte culture [64]. The results indicated that the survival time of hepatocytes cultured on galactosylated gelatin artificial ECM was longer than that of hepatocytes cultured on collagen-coated monolayers, and liver function (e.g., secretion of albumin, synthesis of urea) was maintained due to the specific interaction of galactose moieties in galactosylated gelatin with ASGPR of hepatocytes.

Silk is naturally produced by spiders and Lepdopterai. Silk has been used in ligament tissue engineering due to its unique mechanical properties and good biocompatibility [65]. Gotoh et al. [66] prepared galactosylated silk fibroin by conjugation of lactose with silk using cyanuric chloride as a coupling spacer, and evaluated its ability to facilitate hepatocyte attachment. The results indicated that hepatocyte attachment on galactosylated silk-coated PS dishes increased by approximately eight-fold compared to that of uncoated dishes, and hepatocytes exhibited a round morphology; however, the investigators did not assess alterations to liver function caused by three-dimensional galactosylated silk fibroin.

Xyloglucan, a polysaccharide derived from tamarind seeds, is composed of glucose units in the main chain and xylose and galactose units in the side chains. Seo et al. [67] studied the specific interaction between galactose moieties in the side chain of the xyloglucan and ASGPR of hepatocytes. The results indicated that hepatocyte adhesion to xyloglucan-coated PS surfaces was dependent on the presence of Ca²⁺, and spheroidal hepatocytes formed at a high concentration of xyloglucan in the presence of EGF due to galactose-specific recognition. In addition, alginate microcapsules prepared with xyloglucan as an artificial ECM enhanced multicellular spheroidal hepatocyte formation due to the specific interaction between the galactose moieties of xyloglucan and ASGPR of hepatocytes [68]. Naturally-derived polymers used for liver tissue engineering are summarized in Table 4.

2.3. Parameters affecting hepatocellular behavior

Long-term and stable hepatocyte culture systems are very important for liver tissue engineering and for development of bioartificial liver devices. Morphology, attachment, growth, differentiation, and survival of hepatocytes are highly dependent on galactose density and microdistribution of galactose in galactose-carrying polymers, structure of carbohydrate, topology of ECM, coculture, and cell
source. This section addresses the effects of these parameters on hepatocellular behavior.

2.3.1. Galactose density and microdistribution of galactose

Ligand density is a primary determinant of hepatocyte attachment, morphology, and function; triantennary molecules with three terminal galactose residues bind to lectins with higher affinity than biantennary ones with one or two terminal galactose residues, and a multi-subunit receptor of hepatocytes is responsible for binding galactose residues on desialylated glycoproteins [17].

Kobayashi et al. [30] studied the effects of galactose density in galactose-carrying PS on the morphology and differentiation of hepatocytes. The results indicated that hepatocytes exhibited higher 3H-thymidine uptake (the DNA synthesis indicator) with wider spread morphology at the lower galactose density (0.5/9262 g/ml); however, hepatocytes showed lower 3H-thymidine uptake with a round morphology at higher galactose concentrations (100 µg/ml). The group did not determine an optimal concentration of round hepatocytes. Ise et al. [69] reported that hepatocytes adhered to galactose-carrying PS with coating density lower than 20 ng/ml exhibited higher proliferative ability than those attached to galactose-carrying PS with coating density higher than 50 ng/ml. Yin et al. [37] also reported formation of hepatocyte spheroids one day after cell seeding with better albumin secretion and urea synthesis at a high galactose density of 513 nmol/cm² on the PET surface. Kim et al. [70] proposed a mechanism for hepatocyte behavior according to the coating density of the PVLA (Fig. 5). They explained that ASGPR on hepatocyte membranes are clustered within sites of focal adhesion in a large patch, and that this clustering prevents participation of integrin receptors during the adhesion process at high density. Conversely, hepatocytes allow integrin receptors to participate in the adhesion process within the space where natural ECMs are secreted during cell culture. Thus, inte-

### Table 4

Naturally-derived polymers for liver tissue engineering.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Type of galactose</th>
<th>Linkage</th>
<th>Characters</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>RGD sequence</td>
<td>Amide</td>
<td>Weak mechanical property, Fast enzymatic biodegradation, High cost</td>
<td>[42–45]</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>RGD sequence</td>
<td>Amide</td>
<td>High cost, Coating of fibronectin for application</td>
<td>[46–47]</td>
</tr>
<tr>
<td>Alginate</td>
<td>Lactobionic acid</td>
<td>Amide</td>
<td>Gelation, Easy chemical modification, Unpredictable dissolution of hydrogel in vivo</td>
<td>[58]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Lactobionic acid</td>
<td>Amide</td>
<td>Easy chemical modification, Difficult of control in deacetylation and molecular weight</td>
<td>[59–62]</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Lactobionic acid</td>
<td>Amide</td>
<td>Minimal inflammation, Clinically approved, Weak mechanical properties</td>
<td>[64]</td>
</tr>
<tr>
<td>Silk</td>
<td>Lactose</td>
<td>Ether</td>
<td>Good mechanical properties, Good biocompatibility, Immune reaction by residual sericin</td>
<td>[66]</td>
</tr>
<tr>
<td>Xyloglucan</td>
<td>Galactose</td>
<td>–</td>
<td>Biocompatible, Thermally-reversible gelation</td>
<td>[67–68]</td>
</tr>
</tbody>
</table>
Fig. 5. Illustration of hepatocyte behavior on PVLA surfaces. ASGPR-dependent adhesion occurs initially within the interface between hepatocytes and the PVLA surface at both (A) high coat density and (B) low coat density of PVLA. However, ASGPR-independent adhesion takes place more rapidly at low coat density than at high coat density during culture. It is expected that ASGPR-independent adhesion would be induced by integrin receptors that have the opportunity to participate in cell adhesion mediated by ECMs secreted from hepatocytes following initial adhesion. Finally, the ASGPR-independent adhesion that occurs at low coat density causes hepatocytes to spread through integrin signaling. The concentrated ASGPR-ligand complexes at high coat density inhibit integrin signaling.

Grin receptors play a role in turning round morphologies into spread ones and in focal adhesion kinase phosphorylation due to involvement of the integrin-mediated signal pathway.

The interaction between galactose ligand and ASGPR is not only affected by galactose density on the ECM but also by spatial microdistribution of galactose. Cho et al. [71] studied the effect of orientation on hepatocyte attachment to a PVLA surface prepared by the Langmuir-Blodgett (LB) method. It was found that hepatocytes that adhered to the LB surface of the polymer even at a very low galactose density were round due to the spatial orientation of galactose; these cells were spread on the standard coated PVLA surface. Griffith et al. [34] also reported that spatial microdistribution of galactose in the ECM affected morphology and function of hepatocytes with the accessibility of galactose clustered in spatial microdomains as the hepatocyte receptors organized into aggregated structures within the cell membrane, suggesting that the spatial microdistribution of the ligand in the ECM and control of the microenvironmental niche are important for regulation of cellular behavior and successful tissue engineering.

2.3.2. Topology of artificial ECMs

Topology of artificial ECMs is another important parameter that influences cell morphology, function, and physiological responsiveness due to modulation of cell polarity by the topology of the ECM [72]. It is commonly accepted that three-dimensional artificial ECMs more effectively induce differentiated hepatocyte function than two-dimensional ones due to the provision of better model systems for physiologic situations. Berthiaume et al. [72] reported that hepatocytes cultured in a sandwich configuration of collagen exhibited a dramatic reorganization of the cytoskeleton, adoption of in vivo-like morphology and polarity, and expression of a wide array of liver-specific functions due to the in vivo-like configuration; however, this configuration is difficult to scale-up and has nutrient transport limitations. ECM topology in the micro- and nanometer ranges has been shown to affect cellular behavior, including adhesion, migration, proliferation, and gene expression [73,74]. Ranucci et al. [75] reported that PLGA foams with subcellular size voids (approximately three micrometers) induced two-dimensional hepatocyte reorganization, whereas foams with subcellular size voids (approximately 67 micrometers) promoted three-dimensional aggregation due to enhanced cell-cell contacts. At intermediate void sizes (approximately 17 micrometers), both two-dimensional and three-dimensional reorganization was promoted, and hepatocytes that adhered to collagen foams with a void size of 82 micrometers exhibited a high degree of spreading with high albumin secretion due to three-dimensional intercellular contacts [22].

Park et al. [76,77] reported that hepatocytes cultured on microgrooved glass substrates with a 100 micrometer-high channel between each substrate had significantly better liver-specific function than those in substrates without microgrooves because hepatocytes were protected from
detrimental shear stress and maintenance of oxygen delivery.

Glicklis et al. [78] reported that hepatocytes seeded within three-dimensional porous alginate sponges with diameters of 100–150 micrometers exhibited more than 90% hepatocyte aggregation within 24 h post-seeding due to the non-adherent nature of alginate, and they secreted large amounts of albumin (60 μg/106 cells/day) within a week, even though they did not proliferate.

Recently, a three-dimensional nanofibrous artificial ECM with high porosity and high spatial interconnectivity prepared by electrosprinning has been increasingly applied for tissue engineering [79,80] because its structure is similar to that of natural collagen fibers [81]; and the nanofibrous artificial ECM can promote cell attachment, proliferation, migration, and differentiation, and it can attract more integrin-binding proteins than film artificial ECMs [82]. Chua et al. [83] reported that hepatocytes cultured on galactosylated poly(ε-caprolactone-co-ethyl ethylene phosphate) nanofiber artificial ECMs exhibited cellular function with the enhanced mechanical stability of hepatocyte spheroids similar to that on the two-dimensional galactosylated substrate; however, the cell morphologies were different. Feng et al. [84] reported that hepatocytes cultured on galactosylated chitosan nanofibrous artificial ECMs with an average diameter of 160 nm formed flat, spheroidal hepatocytes and exhibited better liver-specific function than spheroidal hepatocytes on galactosylated chitosan films.

In summary, ECM for liver tissue engineering should be designed to have high galactose density for achievement of higher affinity in hepatocytes, to have three-dimensional artificial ECMs in order to provide better model systems for physiologic situations, and to form hepatocyte spheroids for enhancement of liver-specific function.

3. Cartilage

The connective tissue comprising articular cartilage of the knee is highly specialized for reducing joint friction at the interface of two long bones, and it merely contains chondrocytes in an avascular structure [85]. The regeneration of damaged articular cartilage remains challenging due to its poor intrinsic capacity for repair. Thus far, no surgical procedure has been able to reproduce the biological composition and biomechanical properties of native cartilage [86]. The specific treatment strategies have been dictated by the nature or size of lesions and the preference of the operating surgeon [86]. However, the advent of tissue engineering has provided revolutionary potential for treating cartilage-related diseases. It is believed that tissue engineering of articular cartilage will overcome the current limitations of surgical treatment by offering functional regeneration in the defect region. This technology involves ex vivo culturing of chondrocytes from autologous or allogenic sources in ECM-based constructs and subsequent implantation into the cartilage defect. Although recent progress has been made to engineer artificial cartilage via tissue engineering, the challenges remain significant. Selection of the proper artificial ECMs and incorporation of growth factors or mechanical stimuli is of primary importance to successfully produce artificial cartilage for tissue repair. In this section, artificial ECMs and suitable cell sources used in articular cartilage tissue engineering will be introduced, and growth factors and mechanical stimuli used as cues for inducing differentiation to chondrocytes (Fig. 6) will also be discussed.

Artificial ECMs ideally should furnish chondrocytes with optimal physical conditions mimicking the natural ECM microenvironment of cartilage. Construction of artificial ECMs involves fabrication of a three-dimensional network of artificial ECM similar to that of the original structure and the provision for structural support and internal space for the residing cells to adhere, proliferate, and differentiate. Therefore, general requirements of three-dimensional artificial ECMs include high porosity, controlled degradation, mechanical stiffness and strength, and biocompatibility, and they also should exhibit adequate surface properties for proper tissue formation of chondrocytes [87]. Higher porosity allows for the migration and proliferation of adhering cells and the facilitated exchange of nutrients and waste products. Controlled biodegradability also is important so as not to hinder the formation of newly regenerating tissue within the artificial ECMs. Biocompatibility may facilitate cellular attachment and differentiation.

In most cases, biodegradable polymers including synthetic or natural polymers have been used in cartilage tissue engineering. These polymers usually are formed in sponges, hydrogels, or nanofibers. Recently, composite artificial ECMs composed of a mixture of different polymers were designed to provide chondrocytes residing in the matrix with the combination of merits that each component contributes.

3.1. Natural material-based artificial ECMs

Natural materials have been preferred over synthetic materials because of their intrinsic advantages of biocompatibility, biodegradability, and an improved capacity for cell attachment. They can be divided in two groups: protein-based artificial ECMs (e.g. collagen, fibrin, silk) and carbohydrate-based artificial ECMs (e.g. hyaluronan, alginate, agarose, chitosan). Applications of these natural materials-based artificial ECMs for in vitro and in vivo cartilage tissue engineering are summarized in Table 5.

Collagen is a natural component of cartilage and is known to play an important role in cellular adhesion and differentiation through specific interactions between ligands on collagen chains and adhering cells [86]. Therefore, it is widely used for engineering artificial tissue in a broad spectrum of organs. Type II collagen also plays an essential role in the maintenance of chondrocyte function. Bovine BMSCs seeded on type II collagen represent the most prominent phenotype of chondrocyte differentiation with the addition of transforming growth factor (TGF)-β1 in a time-dependent manner [88]. In addition, chondrogenic differentiation only was detected in three-dimensional hydrogels, not in monolayer cultures. In another in vitro test, it was found that a collagen sponge could provide a superior microenvironment for the formation of ECM, such as proteoglycans, when human intervertebral disc
Fig. 6. Components required for chondrocyte tissue engineering [modified from Ref. [86]].

Table 5
Application of natural materials-based scaffolds for cartilage tissue engineering.

<table>
<thead>
<tr>
<th>Scaffold Component</th>
<th>Forms</th>
<th>Seeding cells</th>
<th>Biofactors</th>
<th>Model/target site</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>A bilayer membrane</td>
<td>Autologous chondrocyte</td>
<td>Human/Deep cartilage defects</td>
<td>Hyaline-like cartilage formation at the defect</td>
<td>[93]</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>A bilayer membrane</td>
<td>Autologous chondrocyte</td>
<td>Sheep/chondral defects</td>
<td>Reparative tissue formation</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td>Fibrin</td>
<td>Hydrogel</td>
<td>Autologous ADSC</td>
<td>Rabbit/chondral defects</td>
<td>Hyaline-like cartilage formation at 8-week follow-up complete healing to subchondral bone</td>
<td>[135]</td>
<td></td>
</tr>
<tr>
<td>Alginate/agarose</td>
<td>Hydrogel</td>
<td>Autologous ADSC</td>
<td>In vitro</td>
<td>Enhanced chondrogenic differentiation</td>
<td></td>
<td>[90]</td>
</tr>
<tr>
<td>Alginate/agarose</td>
<td>Hydrogel</td>
<td>Autologous chondrocyte</td>
<td>Human/osteoenchondral defects</td>
<td>Predominant hyaline cartilage formation at 2-year follow-up Increase in aggregan and type II collagen</td>
<td>[106]</td>
<td></td>
</tr>
<tr>
<td>Alginate</td>
<td>Hydrogel</td>
<td>Allogeneic BMSC</td>
<td>Rabbit/Osteochondral defects</td>
<td>Significantly more hyaline repair tissue formation</td>
<td>[105]</td>
<td></td>
</tr>
<tr>
<td>Chitosan</td>
<td>Hydrogel</td>
<td>Autologous Whole blood</td>
<td>Sheep/Chondral defects</td>
<td>Formation of a more integrated and hyaline repair tissue complete restoration of GAG levels</td>
<td></td>
<td>[108]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Hydrogel</td>
<td>Autologous Whole blood</td>
<td>Rabbit/Bilateral trochlear defects</td>
<td></td>
<td></td>
<td>[109]</td>
</tr>
<tr>
<td>HA</td>
<td>Sponge</td>
<td>Autologous BMSC</td>
<td>FGF</td>
<td>Rabbit/knee osteochondral defect</td>
<td>Formation of cartilage tissue with expressed type II collagen</td>
<td>[101]</td>
</tr>
<tr>
<td>HA esterified</td>
<td>Sponge</td>
<td>Autologous chondrocyte</td>
<td>Human/chronic cartilage lesions</td>
<td>Continued increase of clinical performance No major adverse event during 3-year follow-up</td>
<td></td>
<td>[103]</td>
</tr>
<tr>
<td>derivative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMC</td>
<td>Hydrogel</td>
<td>human nasal chondrocytes</td>
<td>Nude mice/subcutaneous implantation</td>
<td>Formation of a cartilage-like tissue with GAG and type II collagen Sustained release of TGFβ-1 from peptide hydrogel Promoted regeneration of articular cartilage</td>
<td></td>
<td>[110]</td>
</tr>
<tr>
<td>Peptide amphiphile</td>
<td>Hydrogel</td>
<td>hMSC</td>
<td>TGFβ-1</td>
<td>Rabbit/full thickness chondral defect</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HA, Hyaluronic acid; HPMC, hydroxypropyl methylcellulose; PLGA, poly(lactic acid-co-glycolic acid); PEG, poly(ethylene glycol); BMSC, bone-marrow stromal cells; ADSC, adipose-derived stem cells; ESC, embryonic stem cell; FGF, fibroblast growth factor; IGF-1, insulin-like growth factor-1; GAG, glycosaminoglycan; hMSC, human mesenchymal stem cells.
cells were cultured [89]. Artificial ECMs formed from type II collagen were prepared for neo-cartilage synthesis by crosslinking with genipin, because ECM in cartilage is predominantly composed of type II collagen and networks of proteoglycans (PG), such as hyaluronic acid (HA) and chondroitin sulfate (CS). Their study assessed the effect of glycosaminoglycans (GAGs) added to the culture medium on proliferation and matrix synthesis of human chondrocytes [90]. The results indicated that the addition of CS and HA further up-regulated gene expression of aggrecan and collagen II in chondrocytes. In an animal experiment with sheep, cultured autologous chondrocytes seeded in a porcine collagen I-III bilayer membrane were implanted in chondral defects [91]. Microfracture treatment was used to enhance the healing response, and it was reported that combination treatment of microfracture and chondrocyte-seeded collagen membranes further improved the overall cartilage repair. A clinical trial also was performed to check the efficacy of matrix-induced autologous chondrocyte implantation, in which autologous chondrocytes were seeded on three-dimensional membranes of a bilayer type I-III collagen for implantation in deep cartilage defects [92]. The presence of hyaline-like cartilage was observed at the implantation site via magnetic resonance, and chondrocytes and type II collagen also were observed inside the collagen membrane.

The fibrin artificial ECM is a network of fibrous proteins naturally polymerized from fibrinogen in the process of blood clotting in response to injury, and it forms a natural, artificial ECM mesh. It was reported that this three-dimensional structure of fibrin can be used as a gel-type artificial ECM to encapsulate cells for delivery [86]. Several animal experiments were performed using fibrin glue artificial ECMs to evaluate the therapeutic effects of autologous chondrocytes, mesenchymal stem cells, and bioactive molecules on cartilage repair. For autologous adipose-derived stem cells (ADSCs) loaded in a fibrin artificial ECM for implantation in the treatment of full-thickness cartilage defects of rabbits, immunostaining, Western blotting, reverse transcriptase polymerase chain reaction, and quantitative assessment revealed that articular surface defects, treated with an ADSC-loaded artificial ECM, healed with hyaline-like cartilage; however, very little healing was observed in the control group. Cartilage markers including aggrecan and collagen type II mRNA also were identified in the treated group. However, concerns regarding immunological reactions have been raised; if autologous fibrin is not used, the method requires additional procedures and time necessary for blood collection [93].

Silk is an artificial ECM that has been studied by several groups because of its high mechanical strength in the wet state and its low inflammatory properties [94]. It also was reported that fibroin, a primary component of silk, demonstrates good cell attachment and biocompatibility. Wang et al. reported that human chondrocytes seeded in silk fibroin artificial ECMs could be re-differentiated after expansion on culturing dishes and deposited in cartilage-specific ECM for in vitro cartilage tissue engineering [95]. In another in vitro experiment, better-defined cartilage tissue with increased GAG amounts and cell density were observed in silk fibroin sponge hydrogels compared to that of collagen gel [96]. These characteristics also were observed with bone marrow-derived mesenchymal stem cells (BMSCs) seeded on silk fibroin artificial ECMs. In particular, BMSCs proliferated more rapidly on the silk fibroin artificial ECM, and homogeneous distribution of cartilage-like tissue was achieved only with silk artificial ECM samples among silk, collagen, and cross-linked collagen artificial ECMs. Use of exogenous silk protein for tissue engineering might be problematic due to its stimulation of immune responses, such as induction of significant TNF release from macrophages [98].

Hyaluronan is a primary physiological component of ECMs in articular cartilage, which can be chondrogenic to mesenchymal stem cells. In a transplantation study with autologous BMSCs embedded in a hyaluronic acid sponge, BMSC-loaded artificial ECMs were implanted in full-thickness osteochondral defects of the rabbit knee [99]. Histological findings revealed that newly formed cartilage tissue at the implantation site was very similar to the surrounding normal tissue, which is much better than that of the untreated group. It is usually chemically modified for easy fabrication into solid artificial ECMs because of its highly hydroscopic nature [100]. The most common form of the modification is the esterification of a carboxylic acid present at the C6 position of hyaluronic acid. The benzyl ester of hyaluronic acid has been commercialized as Hayff-11 and tested clinically. Autologous chondrocytes isolated from patients were seeded on Hayaff-11 and implanted in the chronic cartilage lesions of their knees [101]. There were no major adverse events reported during three-year follow-up, and clinical outcomes were prominent with the treated group. However, the chemical modification of hyaluronic acid might compromise its biocompatible property. In addition, the degradation of cross-linked hyaluronan caused chondrolysis [102].

Alginate is an anionic polysaccharide, which forms a hydrogel instantly in the presence of divalent cations, such as calcium ions. Alginate beads encapsulating cells are commonly produced when cells dispersed in alginate solution are dropped into a calcium chloride solution. In an in vitro study using rabbit BMSCs, it was reported that alginate and agarose gels containing BMSCs induced a greater increase in the expression of cartilage-specific markers, such as aggrecan and type II collagen, than cells in type I collagen gels [103]. Furthermore, when rabbit BMSCs encapsulated in alginate beads were deployed in the cartilage defects of rabbit, the beads remained satisfactorily within the defect regions, which were progressively replaced by the regenerating tissue. Histological findings showed that viable chondrogenic cells filled in the defects. In a clinical study following 17 patients (inclusion criteria: an isolated lesion of the femoral condyle (grades III and IV)), significant improvement was observed in patients with lesions larger than 3 cm² when autologous chondrocytes were isolated and suspended in an alginate-agarose mixture solution and subsequently implanted in the lesions of patients [104]. However, alginate gel suffers from instability in physiological solution due to a loss in mechanical strength and integrity induced by the replacement of divalent calcium ions with monovalent sodium or potassium ions.
Chitosan also is preferred in articular tissue engineering applications because of its structural similarity to GAG components in cartilage-stimulating chondrogenesis [105]. In a study characterizing cartilage repair in sheep, a chitosan-glycerol phosphate solution was mixed with autologous whole blood from sheep to facilitate the healing process in cartilage defects with blood clots [106]. Interestingly, the mixture of chitosan-glycerol phosphate and whole blood showed increased adhesion to walls of the cartilage defect region and significant repair in hyaline cartilage at a 6-month follow-up. In a subsequent study with a rabbit model, it was reported that beneficial blood clot formation could be stabilized by chitosan-glycerol phosphate solution via inhibition of clot retraction [107]. Since chitosan possesses primary amino groups at the C2 position of each repeating unit, which is only protonable below pH 6.5, this unique characteristic allows for convenient processing in artificial ECMs under mild conditions. The polycationic property of chitosan can be exploited to form a more stable ionic complex with polyanionic macromolecules including alginate, GAGs, synthetic poly(acrylic acid), and smaller anionic molecules. Chitosan microspheres were fabricated by drop-wise addition of anionic tripolyphosphate in a chitosan emulsion containing TGF-β1 for the tissue engineering of articular cartilage [108]. Controlled release of a bioactive growth factor was achieved from ionically crosslinked chitosan microspheres, and in vitro culture of porcine chondrocytes on the microspheres demonstrated a significant increase in cell proliferation and ECM production.

Recently, a novel peptide amphiphile (PA) with a TGF-β1-binding peptide domain was explored for cartilage regeneration applications [109]. This self-assembling PA formed supramolecular nanofibers upon the addition of charge-shielding ions, which resulted in a hydrogel displaying a high density of exposed TGF-β1-binding peptide for efficient capture and release of TGF-β1 growth factor. When human mesenchymal stem cells (hMSCs) were suspended in PA solution and injected in a full-thickness, articular cartilage rabbit model with microfracture, the data demonstrated improved survival and increased chondrogenic differentiation of hMSCs. Furthermore, PA itself, even without TGF-β1 growth factor, promoted the regeneration of articular cartilage in a full-thickness chondral defect, suggesting the possibility that TGF-β1-binding domains in PA might serve as a reservoir for attracting and releasing the endogenous TGF-β1.

### 3.2. Synthetic material-based artificial ECMs

Synthetic materials including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymer, poly(lactic acid-co-glycolic acid) (PLGA), have been tested for cartilage tissue engineering potential (Table 6). They are thought to have advantages including ease in molding and the ability to design a degradation rate to match tissue growth into the artificial ECM. However, these synthetic materials are not as preferred for cartilage tissue engineering as materials of natural origin, because the acidic byproducts generated during the degradation process cause an inflammation reaction, giant cell reaction, and acute chondrocyte death.

<table>
<thead>
<tr>
<th>Scaffold component</th>
<th>Synthetic materials-based scaffold</th>
<th>Seeding cells</th>
<th>Biofactors</th>
<th>Model/target site</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>PEG</td>
<td>Crosslinked hydrogel</td>
<td>Bovine chondrocytes</td>
<td>TGF-β1</td>
<td>Nude-mouse/obtuseaneous implantation in vivo; Gene expression of cartilage-related markers mature elastic cartilage found after 10 weeks</td>
</tr>
<tr>
<td>PEG</td>
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<td>TGF-β1</td>
<td>Nude-mouse/obtuseaneous implantation in vivo; Gene expression of cartilage-related markers mature elastic cartilage found after 10 weeks</td>
</tr>
<tr>
<td>p(NiPAAm-co-AAc)</td>
<td>PEG</td>
<td>Thermo-responsive hydrogel</td>
<td>Bovine chondrocytes</td>
<td>TGF-β1</td>
<td>Nude-mouse/obtuseaneous implantation in vivo; Gene expression of cartilage-related markers mature elastic cartilage found after 10 weeks</td>
</tr>
<tr>
<td>PLGA/fibrin</td>
<td>PLGA/fibrin</td>
<td>Sponge</td>
<td>Rabbit chondrocyte</td>
<td>TGF-β1</td>
<td>Nude-mouse/obtuseaneous implantation in vivo; Gene expression of cartilage-related markers mature elastic cartilage found after 10 weeks</td>
</tr>
<tr>
<td>MPEG-PLGA/fibrin</td>
<td>MPEG-PLGA/fibrin</td>
<td>Sponge</td>
<td>Rabbit chondrocyte</td>
<td>TGF-β1</td>
<td>Nude-mouse/obtuseaneous implantation in vivo; Gene expression of cartilage-related markers mature elastic cartilage found after 10 weeks</td>
</tr>
</tbody>
</table>
due to the abrupt drop in pH in the local microenvironment inside the engineered cartilage. The closed cartilage environment deters rapid clearance of acidic byproducts from the degrading synthetic artificial ECMs, eventually inducing undesirable side reactions in cartilage. Another disadvantage of these materials is their poor cell attachment. Commonly, these materials were modified to possess an anchorage site for cell adhesion or mixed with other naturally-derived materials [110].

3.2.1. Poly(ethylene glycol) (PEG) hydrogels

Recently, hydrogel-based artificial ECMs have gained greater attention for cartilage tissue engineering applications because of their similarity to the natural cartilage environment. Hydrogels contain high water content similar to that of natural cartilage, which serves as a suitable environment for chondrocytes. These materials are composed of synthetic or natural-based hydrophilic biomaterials cross-linked by physical, ionic, or chemical interactions. They also can be injected transcutaneously into the defect region of the joint, which avoids invasive surgery required for the implantation of prefabricated artificial ECMs. PEG is a popular biocompatible hydrophilic polymer approved by the FDA, and it has been extensively explored for the use of formulating hydrogels to encapsulate bioactive drugs or cells. It is thought that crosslinked PEG hydrogel may provide a better environment for culturing chondrocytes due to its high water content and mechanical strength, because chondrocytes are surrounded by hydrophilic ECM components in high abundance. In one approach to minimize invasiveness during treatment of the cartilage defect while maintaining its mechanical and biological properties, photopolymerization was employed using a PEG macromer polymerizable at both ends and a photoinitiator, followed by exposure of the solution to UV light [111]. Photopolymerized PEG hydrogel is an attractive material for tissue engineering applications because of its cell-friendly properties and in situ gelation upon exposure to UV. In this study, a PEG macromer was mixed with isolated allogenic chondrocytes in the presence of a photoinitiator. The photopolymerization reaction can be performed at physiological pH and temperature, which allows for safe encapsulation of cells and proteins. Chondrocyte viability in this hydrogel was maintained with characteristic gene expression for up to three days of in vitro culture. In another study, cell binding peptide was conjugated to one end of a bifunctional PEG macromer and mixed with PEG diacrylate in the presence of a photoinitiator for crosslinking. When BMSCs and embryonic stem cell-derived cells (ESCs) were encapsulated in this hydrogel with a mechanical stimulus, gene expression of cartilage-related markers, such as Sox-9, type II collagen, and aggrecan, was noticed [112]. However, it was determined that highly crosslinked PEG hydrogel might hinder the proliferation and proteoglycan synthesis of encapsulated chondrocytes [113,114]. As the seeded cells grow and form new tissue inside the hydrogel, the scaffold should degrade accordingly. The effect of the degradation rate of hydrogel on the engineered cartilage formation was evaluated using hydrogels of different degradation rates, which were prepared by copolymerizing a degradable PLA-b-PEG-b-PLA macromer with non-degradable PEG macromers at pre-determined mixing ratios [115]. It was found that chondrocytes encapsulated in a PEG hydrogel with a higher degradation rate produced more DNA content and type II collagen, indicating the important role of hydrogel degradation in controlling and influencing the deposition and distribution of ECM molecules. The photopolymerized PEG hydrogel also can be functionalized for cellular anchorage by incorporating a cell adhesion peptide, such as Arg-Gly-Asp (RGD), at PEG chain terminals during polymerization [116].

3.2.2. Injectable hydrogels

In situ injectable hydrogel systems have generated increased interest for cartilage repair applications. They can be injected with encapsulated cells and/or bioactive materials of interest into the cartilage defect in a minimally invasive manner and easily can fill the three-dimensional shape of the defect for facilitated integration with the additional mechanical property of temperature-dependent sol-gel transition. Poly(N-isopropylacrylamide) (PNIPAAm) exhibits reversible phase separation with a lower critical solution temperature (LCST) of approximately 32°C. Thus, chondrocytes cells can be dispersed in PNIPAAm solution at room temperature (RT) lower than LCST and injected into the cartilage defect for in situ gelation. When isolated bovine articular chondrocytes were seeded into the loosely crosslinked copolymer hydrogel of NIPAAm and acrylic acid, (p(NIPAAm-co-AAc), and incubated at 37°C in vitro, the viability of seeded chondrocytes was maintained for up to four weeks, and cartilage-like tissue formation was observed in the matrices [117]. In an in vivo study using this thermo-reversible hydrogel, a larger volume of cartilage-associated ECM was observed with the addition of dexamethasone, ascorbate, and TGF β-3 eight weeks after subcutaneous implantation in nude mice [118]. However, the temperature-dependent phase transition might hinder the thermo-responsive gel from being injected through a long needle or catheter to the desired site in the human body, because the warmed needle or catheter can eventually cause premature gelation during injection before reaching the target site. In another approach to inhibit this pre-mature gelation, a p(NIPAAm-co-vinylimidazole) [p(NIPAAm-co-VI)] copolymer hydrogel that responds to both temperature and ionic strength was used [119]. Rabbit chondrocytes were embedded in this composite hydrogel with TGF β-1-loaded nanoparticles. Chondrocyte-specific ECMs were observed eight weeks after subcutaneous implantation of the hydrogel in nude mice.

Pluronic F-127 consists of approximately 70% ethylene oxide and 30% propylene oxide by weight and is available commercially [120]. It transforms to hydrogel at RT at concentrations of 20% or higher. After administration in vivo, it can be slowly dissolved and cleared by renal and biliary excretion. Although several trials have been conducted using Pluronic F-127, poor mechanical properties hinder its application for tissue engineering due to a difficulty maintaining the constructed shape. Thus, composite artificial ECMs that compensate for the poor mechanical properties of Pluronic F-127 have been preferred, which will be described later.
3.3. Composite artificial ECMs

A few specific characteristics of single ECM components might be insufficient to create the optimal environment to mimic the natural proliferation and differentiation of chondrocytes in cartilage. Therefore, in most cases a combination of multiple components is desired to address various features required for culturing chondrocytes. Most synthetic material-based artificial ECMs suffer from poor anchorage of chondrocytes or stem cells, and incorporation of natural ECMs including collagen, fibronectin, and hyaluronic acid in synthetic artificial ECMs for cell attachment has been a popular approach studied extensively. Synthetic materials provide relatively high mechanical strength with a tunable degradation rate, whereas their hydrophobicity and lack of cellular anchorage sites are drawbacks for their application in tissue engineering. Conversely, naturally-derived ECM polymers support excellent cellular adhesion and growth due to their specific cell interaction peptides and hydrophilicity, even though their weak mechanical properties make it difficult to use them in load-bearing regions, such as cartilage. Thus, these characteristics of naturally- or synthetically-originate materials drive us to attempt a combination of the two materials in order to afford higher mechanical strength, tunable degradation, and cellular attachment [121]. When collagen microsponges were embedded in a PLGA mesh and transplanted subcutaneously into nude mice for chondrocyte tissue engineering, homogeneous cell distribution, natural chondrocyte morphology, and abundant cartilaginous ECM deposition were observed. As a biomimetic approach, the active cell anchorage domain of collagen (e.g. RGD) was conjugated with the surface of synthetic material-based artificial ECMs [122]. Chondrocytes seeded on RGD-modified PLGA/gelatin microspheres demonstrated the best attachment, proliferation, viability, and sulfated GAG secretion.

Another purpose of hybrid artificial ECMs is to incorporate thermally or chemically responsive components in natural or synthetic material-based artificial ECMs to increase the bioavailability of seeded cells while minimizing their leakage out of the artificial ECMs. Fibrin possesses a chemically active gelling property, which typically occurs during blood coagulation in addition to the advantages described previously. Several studies investigating a fibrin-based hybrid artificial ECM demonstrated its potential for the promotion of homogeneous cell distribution and cartilaginous tissue formation [110,123,124]. PNIPAAm was also used for this purpose, and it is a synthetic thermo-responsive polymer for tissue engineering with versatile manipulation of its chemical structure and molecular weight as well as easy conjugation with other components. Chondrocytes could be seeded easily into the PLA-PNIPAAm hybrid artificial ECMs at RT, at which the hydrodynamic structure of PNIPAAm was shrunk to create larger pore sizes for facile cell loading [125]. Upon incubation at 37 °C, the PNIPAAm was rehydrated to provide a more hydrophilic and chondrocyte-friendly microenvironment, and its swelling property at physiological temperature in PBS solution was maintained for 4 weeks. Chondrocytes cultured in this hybrid artificial ECM could be harvested easily from the artificial ECM by simply lowering the temperature to RT without any additional treatment by proteolytic enzymes, and the harvested chondrocytes exhibited a round morphology, indicative of maintenance of the chondrocyte phenotype during culture in the hybrid artificial ECM.

4. Bone

4.1. Artificial ECMs for bone tissue engineering

4.1.1. Design criteria of artificial ECMs for bone tissue engineering

A key component in bone tissue engineering is an artificial ECM that serves as a template for cell interactions and the formation of bone ECM to provide structural support for the newly formed tissue. The artificial ECMs for bone tissue engineering should meet several criteria to serve this function including possession of mechanical properties similar to those of the bone repair site, biocompatibility, biodegradability at a rate commensurate with remodeling, and porosity of artificial ECMs that allows migration and proliferation of osteoblasts and mesenchymal cells as well as vascularization. In addition to these criteria, artificial ECMs should be able to regulate cellular functions even though several reviews have focused on artificial ECM properties including mechanical properties [126,127], degradation [128,129], porosity [130], and osteoconductive activity of the artificial ECM [131,132] for bone tissue engineering. In this section, we reviewed studies that focused on the regulation of cellular function by artificial ECMs after the introduction of materials used in bone tissue engineering. In addition to polymeric materials, non-polymeric materials were also used to fabricate artificial ECMs for bone tissue engineering because bone is a hard tissue.

4.1.2. Non-polymeric materials

Non-polymeric materials including metallic and ceramic materials have been widely used for bone tissue engineering. Generally, the mechanical properties of metallic materials (e.g. stainless steel, cobalt–chrome based alloys, titanium (Ti) alloys) are superior to those of polymeric ones. However, there are several disadvantages, such as the lack of tissue adherence, low rate of degradation, toxicity due to accumulation of metal ions due to corrosion, and mismatch of Young’s moduli between metallic materials and bone [133–135]. Furthermore, the Ti alloys are not osteoconductive [136]. Ceramic materials such as calcium phosphate also have been used extensively in bone tissue engineering because they are natural components of bone tissue [137–140]. Calcium phosphate can be used in permanent implants or as biodegradable artificial ECMs due to its osteoconductive and osteoinductive properties as well as mechanical compatibility with native bone. Although calcium phosphate has several attractive properties, its mechanical properties are poor [126]. Therefore, its clinical use is limited to non-weight bearing sites.
4.1.3. Synthetic polymeric materials

Compared to metallic and ceramic materials, polymeric materials are materials in which it is easy to chemically incorporate moieties that regulate cellular functions. Also, synthetic polymers can be produced under controlled conditions and, therefore, exhibit predictable and reproducible mechanical and physical properties, such as tensile strength, elastic modulus, and degradation rate. Moreover, three-dimensional, biodegradable, synthetic polymeric systems are of particular interest because their porosity, hydrophilicity, and degradation time can be varied with a high degree of reproducibility. For bone tissue engineering, there are several commonly used synthetic polymers, such as PLA, PGA, poly(e-caprolactone) (PCL), and PLGA [100,140–143]. To fabricate these polymers into three-dimensional artificial ECMs, there are several techniques including salt-leaching, gas foaming, electrospinning, and emulsion polymerization [130]. PLGA-based artificial ECMs fabricated via consolidation by pressure drop have been used as teeth implants [141]. PLGA/poly(vinyl alcohol) blend fabricated via a salt-leaching technique were used as an artificial ECM for skull bone defect treatment [142] even though they have been applied in areas of low mechanical stress in vivo because of their low mechanical properties [143].

4.1.4. Naturally-derived polymers

Naturally-derived polymers (e.g. collagen, hyaluronic acid, glycosaminoglycans) have the advantages of biocompatibility and biodegradability because they are composed of the structural materials of tissues. Kim et al. used hyaluronic acid-based hydrogels containing bone morphogenetic protein-2 (BMP-2) and human mesenchymal stem cells to treat a rat calvarial defect model [144]. Collagen-based artificial ECMs also have been used for bone tissue engineering [145,146]. Type I collagen is a major component of in vivo bone ECM and leads to new bone from stem/progenitor cells via the developmental cascade. Therefore, type I collagen might be a good candidate material for a biomimetic approach to design artificial ECMs for bone tissue engineering. In addition to hyaluronic acid and type I collagen, silk fibroin also has been used as the artificial ECM in bone tissue engineering [147]. Salt-leaching techniques and freeze-drying techniques are commonly used to fabricate these materials. Since they are generally mechanically weak, it might be difficult for natural materials to be used as the bone tissue engineering artificial ECMs by themselves even though they have high biocompatibility and biodegradability.

4.1.5. Composite materials

Even though each individual material has advantages for bone tissue engineering applications, each material discussed has disadvantages in various properties including the brittleness of calcium phosphate and the inferior mechanical properties of natural and synthetic polymers. The combination of different materials to form composites has led to overcoming the disadvantages of any one particular material. For example, to develop Ti-based artificial ECMs with osteoconductive activity, artificial ECM surface was coated with a hydroxyapatite layer which improved interactions with osteoblastic cells and promoted osteogenic activity (e.g. osteocalcin expression) in vitro compared to that of a non-coated Ti-based artificial ECM [148]. Brittleness of calcium phosphate is a major problem for its implementation as an artificial ECM for bone tissue engineering. A hydroxyapatite-based artificial ECM was coated with a hydroxyapatite/PCL composite and demonstrated increased compressive strength and elastic modulus compared to an uncoated hydroxyapatite-based ECM [149]. In addition, a collagen/hydroxyapatite composite artificial ECM was developed to support the attachment and proliferation of rabbit periosteal cells [150]. Furthermore, a PLGA sponge hybridized with collagen microsponges on which apatite particulates were deposited is an example of a three-component artificial ECM for bone tissue engineering [151].

4.2. Regulation of cellular functions by artificial ECMs

4.2.1. Importance of regulation of cellular function by artificial ECMs

When materials were used for bone regeneration, the sole criterion was “to achieve a suitable combination of physical properties to match those of the replaced bone tissue with a minimal toxic response of the host” [152,153]. However, all bone tissue functions cannot be mimicked by the materials currently used for bone regeneration because they are unstable and cannot work well in vivo after long periods of implantation. To reconstruct new bone tissue, regulation of cellular function is necessary. There are several approaches, such as the application of growth factors and mechanical stimuli that in turn regulate cellular function. In addition, artificial ECMs play a central role in the regulation of cellular function because the artificial ECMs respond to growth factors and transmit mechanical stimuli. Therefore, the design of artificial ECMs will become a primary issue for the regulation of cellular function for bone tissue engineering applications in the future. For bone tissue engineering applications using cells, osteoblasts and mesenchymal stem cells (MSCs) are important cell sources. In this section, we primarily discussed materials for the regulation of osteoblast function. The materials for bone tissue engineering using MSCs are reviewed in Section 6.

4.2.2. Regulation of osteoblast functions

To regulate cellular function using artificial matrices, mimicking native ECM is a well established, reliable approach. To mimic native ECM in bone, ECM proteins secreted from osteoblasts or MSCs are used for the regulation of cell function [154–158]. Using these materials, it is possible to regulate cellular functions, such as proliferation and differentiation. To mimic native bone ECM more efficiently, fragments of the ECM proteins are incorporated within polymeric materials. Type I collagen has been used widely as an artificial ECM, mimicking in vivo ECM in bone tissue engineering applications. The osteoblasts cultured on type I collagen matrix expressed an osteoblastic phenotype at higher levels than those cells on plastic surfaces [159]. These cells recognize type I collagen by the cell surface receptor, integrin α2β1 [160].
where the integrin receptor activated intracellular signaling pathways to express the osteoblastic phenotype. To confirm this mechanism, synthetic peptides also have been used. When integrins bind to the ECM molecules, integrins recognize the biologically significant peptide region, Arg-Gly-Asp (RGD). Hu et al. developed an RGD peptide-modified PLA film [161] on which they demonstrated enhanced osteoblast attachment. Also, alkaline phosphatase activity and calcium deposition increased on this film, indicating that the osteogenic phenotype was expressed. In addition to the RGD peptide, a collagen peptide motif also was applied to the osteoblast culture [162]. The ECM also regulated the activity of cytokines by increasing their accessibility to receptors [155,156]. Therefore, the incorporation of cytokines is one of the most useful approaches for regulating cellular function [163,164].

4.3. Artificial ECMs for osteochondral defects

Osteochondral defects are often associated with mechanical instability of the joint and, therefore, with the risk of causing osteoarthritic degenerative changes. Grafting of osteochondral units with both chondral and osseous layers represents a promising approach to restore the biological and mechanical functionalities of the joint. To transplant osteochondral units, autologous osteochondral grafts (i.e. mosaicplasty technique) are currently used clinically; however, this technique suffers from several limitations, such as lack of availability of materials, donor site morbidity, and difficulty in matching the topology of the grafts with the injured site. Therefore, osteochondral composites have been developed to overcome these limitations. Although homogenous artificial ECMs were often fabricated for the reconstruction of osteochondral composites [165,166], the materials were designed to produce cartilaginous and bone parts separately [167–170]. Because cartilage and bone are different tissues, the material design should be optimized accordingly for each tissue. To fabricate osteochondral composites, two approaches have been utilized: (1) cartilage and bone tissues are independently reconstructed and combined into a single composite graft by suturing or adhering them together, and (2) cartilage and bone tissues are reconstructed in multi-phasic artificial ECMs.

For fabricating a single phase of each tissue layer followed by reconstruction in osteochondral composites, several approaches that were optimized for each tissue have been utilized. These materials have been discussed in detail previously. For example, periosteal-derived cells were cultured in a PLGA and PEG hybrid artificial ECM to generate reconstructed bone. The cells in the PLGA/PEG artificial ECM deposited mineralized matrix and bone specific proteins (e.g. osteocalcin, osteopontin). The engineered bone was sutured together with reconstructed PGA-based engineered cartilage seeded with articular chondrocytes [167]. Similarly, collagen-hydroxyapatite hybrid sponges were used as the bone component of osteochondral composites, and the artificial ECMs were sutured to the engineered cartilage [168].

In addition to the physical combination of independently engineered cartilage and bone tissues, reconstruction of osteochondral composites in a multi-phasic artificial ECM has been explored. Scheck et al. developed a biphasic artificial ECM composed of PLGA and HA. At the interface between PLGA and HA, a thin PGA film was deposited to prevent cell migration between the components [169]. Porcine articular chondrocytes were seeded in the PLA region of this artificial ECM (cartilage layer), while human gingival fibroblasts transduced with an adenovirus expressing BMP-7 were seeded in the HA region of the artificial ECM (bone layer). Following ectopic implantation in mice, the cartilage layer contained ECM rich in glycosaminoglycans. In contrast, blood vessels were observed in bone layer. Moreover, a mineralized interface was often present at the junction. Kon et al. also investigated multilayered gradient artificial ECMs composed of HA and type I collagen [170]. They divided the artificial ECM into three parts (cartilage, transition, and bone regions). According to their results, the compositions of type I collagen and HA varied in each region (cartilage region: 100% type I collagen; transition region: 40% HA and 60% type I collagen; and bone region: 70% HA and 30% type I collagen). In this case, chondrocytes were seeded only in the cartilage region and were transplanted in the osteochondral defect model. The regeneration of bone and cartilage was significantly enhanced with the artificial ECMs compared to that of the control group.

In summary, the incorporation of bioactive molecules is a good approach for regulating cellular function. The optimization of physical properties (e.g. elasticity, porosity of three-dimensional artificial ECMs) also is required to regulate cellular function precisely for bone tissue engineering applications.

5. Skin

Several tissue-engineered skin products are commercially available. The application of tissue-engineered skin is expanding from laboratory use (e.g. skin biology research, drug evaluation) to clinical use [171]. Although tissue-engineered skins are commercially available, skin tissue engineering research should progress to include additional applications. Several types of artificial ECMs composed of naturally-derived ECM proteins and synthetic polymeric materials have been used to develop engineered skin [172–179].

Skin tissue is divided into two parts: epidermis and dermis. In the epidermis, keratinocytes are the most common cell type and form a surface barrier layer. In the dermis, fibroblasts exist in skin ECM and provide strength and resilience. Most tissue-engineered skins are created by expanding skin cells and using them to restore barrier function or to initiate wound healing. Many tissue engineered skin dermal replacement products contain artificial ECMs as a barrier or substratum for skin cells. The artificial ECM must eventually be discarded or replaced by live skin cells for long-term healing, but artificial ECM can be used temporarily to provide a barrier or a substratum for the cells. Here, we summarized artificial ECMs used in the skin tissue engineering field.
For dermal replacement, several naturally-derived ECM proteins have been used as artificial ECMs for skin tissue engineering. Type I collagen has been used extensively in this application since it is a major component of dermis ECM. Integra (produced by Integra Life Sciences) is an example of type I collagen-based artificial ECM for skin tissue engineering [172]. Type I collagen-based artificial ECM also has been developed to replace epidermis and dermis [173,174]. Apligraf (produced by Organogenesis) is composed of keratinocytes and fibroblasts with type I collagen-based artificial ECM. In addition to type I collagen, fibrin (BioSeed-S) and hyaluronic acid (Hyalograft-3D) have been used as an artificial ECM for skin tissue engineering [175]. In addition, acellular dermis from cadaveric skin (Alloderm) has been used as an artificial ECM to mimic the in vivo ECM of skin tissue [176].

5.2. Synthetic polymeric materials

Synthetic polymeric materials have also been used for skin tissue engineering applications. Bioresorbable PLGA (polyglactin 910) is used in Dermagraft which is a commercially available skin substitute [177]. Furthermore, other synthetic polymeric materials, such as PCL and PLGA, have been used for a long time as artificial ECM in skin tissue engineering [178,179]. Table 7 summarizes the characteristics of polymers used in skin tissue engineering.

5.3. Future challenges in skin tissue engineering

Although tissue-engineered skins are commercially available, there are several challenging issues including the improvement of safety and angiogenesis. In addition to these issues, reconstruction of more complex structures in skin is expected because in addition to epidermal and dermal tissues there are hairs, sweat glands, and touch receptors in native skin tissues. Therefore, it is necessary to develop engineered skin with these structures in the future.

6. Stem cells

Artificial ECMs can modulate stem cells’ behavior, including adhesion, proliferation and differentiation. Soluble factors, cell adhesion signals and mechanical signals can be incorporated into artificial ECMs for the control of stem cell behavior. The chemical and physical properties of artificial ECMs, such as material chemistry, architecture and mechanical properties can also modulate stem cells’ behavior, which is summarized in Table 8.

6.1. Delivery of soluble factors using artificial ECMs

Soluble factors, including growth factors and hormones, play a significant role in the modulation of stem cell behavior. Incorporation or local delivery of certain growth factors using artificial ECMs may increase the accessibility of said factors to seeded stem cells as well as prevent growth factor loss due to diffusion, which would be an effective way of directing stem cell behavior. Artificial ECMs can retain and present growth factors via direct entrapment, affinity and covalent tethering.

Growth factors can be directly entrapped in artificial ECMs. Moreover, the release of growth factors directly entrapped in artificial ECMs or delivery systems is generally controlled via diffusion through or degradation of delivery systems, or even a combination of these two mechanisms [180,181]. One example of stem cell control by the growth factor delivery is that vascular endothelial growth factor (VEGF) delivery using a hydrogel can induce vascular differentiation of human embryonic stem cells (ESCs) entrapped in the hydrogel [182]. Another example is that insulin-like growth factor-1 and transforming growth factor-β1 (TGF-β1) released from gelatin microparticles entrapped in oligo(poly(ethylene glycol)fumarate) hydrogel can induce chondrogenic differentiation of mesenchymal stem cells (MSCs) entrapped in the hydrogel [183].

An affinity-based delivery system can release growth factors retained in a delivery system via non-covalent interactions. Heparin-conjugated artificial ECMs have been developed for the local release of growth factors with affinity to heparin. The delivery system is based on the inherent capacity of heparin to bind to growth factors such as fibroblast growth factor 2 (FGF2), VEGF, platelet-derived growth factor (PDGF), TGF-β and bone morphogenetic protein (BMP) via electrostatic interactions between the negatively-charged sulfate groups of heparin and the positively-charged amino acid residues of the growth factor proteins [184,185]. Such a system can deliver FGF2, which allows long-term self-renewal [186] and pluripotency maintenance [187] of human ESCs. Heparin was also conjugated to poly[lactic-co-glycolic acid] (PLGA) scaffolds for BMP-2 delivery, creating a system capable of potentiating the osteogenic efficacy of BMP-2 [188]. Local delivery of BMP-2 using this system induced in situ osteogenic dif-

### Table 7

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Name in market</th>
<th>Application</th>
<th>Reference</th>
</tr>
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<tr>
<td>Collagen</td>
<td>Integra</td>
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<td>[172]</td>
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<tr>
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<td>Epidermal/dermal replacement</td>
<td>[173]</td>
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<tr>
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<td>Cincinnati skin substrate</td>
<td>Epidermal/dermal replacement</td>
<td>[174]</td>
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<td>Bioseed-S</td>
<td>Epithelial cover</td>
<td>[175]</td>
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<tr>
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<td>Hyalograft-3D</td>
<td>Dermal replacement</td>
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<td>Alloderm</td>
<td>Dermal replacement</td>
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<tr>
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<td>Dermagraft</td>
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<td>Synthetic polymers</td>
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<tr>
<td>PLGA</td>
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Table 8
Modulation of stem cells’ behavior with interactions with signals from artificial ECMs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Artificial ECM</th>
<th>Stem cell</th>
<th>Stem cell's behavior</th>
<th>References</th>
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</tbody>
</table>

Growth factors can be retained on artificial ECMs via covalent tethering. Recently, a study showed that a growth factor tethered to an artificial ECM exhibits higher activity than soluble growth factor [190]. The authors hypothesized that the cellular response to tethered growth factor would be different due to cellular binding to the growth factor on a culture surface, which would inhibit internalization of the growth factor into the cytoplasm. MSCs were cultured on an epidermal growth factor (EGF)-tethered poly(methyl methacrylate)-graft-poly(ethylene oxide) (PMMA-g-PEO) surface or a PMMA-g-PEO surface with EGF supplemented to the culture medium. EGF is known to promote MSC proliferation and migration without affecting pluripotency [191]. Surprisingly, tethered EGF was superior to EGF in solution for the promotion of MSCs spreading and survival in the presence of Fas ligand, a potent cell death factor. The authors concluded that EGF-tethered artificial ECM may offer a protective advantage over MSCs seeded on artificial ECMs transplanted in undifferentiated state [189].
ECM in vivo if the artificial ECM causes an acute inflammatory response detrimental to the MSCs.

6.2. Cell adhesion signal presentation by artificial ECMs

Conjugation of cell adhesion peptides to artificial ECMs can promote stem cell differentiation. Previously, RGD peptides were covalently conjugated to PEG diacrylate hydrogels using conventional N-hydroxysuccinimidyl-ester (NHS) chemistry. It was found that functionalization of PEG diacrylate hydrogels promotes the osteogenic differentiation of human MSCs [192]. Another study showed that RGD peptide incorporation into oligo(PEG-fumarate) hydrogels promotes osteogenic differentiation of MSCs [193]. The osteogenic differentiation of MSCs on peptide-modified oligo(PEG-fumarate) hydrogels is dependent on the concentration of incorporated peptide: the ALP activities of MSCs in hydrogels modified with 2.0 and 1.0 μmol peptide/g were significantly greater compared to MSCs in hydrogels modified with 0.1 μmol peptides/g or no peptide. The behavior of bone marrow stromal osteoblasts cultured in hydrogels modified with two types of peptides, rat osteopontin-derived peptide and RGD, were compared previously [194]. The results showed that hydrogel modified with osteopontin-derived peptide exhibits significantly higher cell migration than RGD-modified hydrogels. RGD peptide-conjugated PEG diacrylate hydrogels also promote the chondrogenic differentiation of human ESCs [195]. Compared to human ESCs in PEG diacrylate hydrogels, cells in RGD peptide-conjugated PEG diacrylate hydrogels exhibit enhanced gene expression of cartilage-specific markers (i.e., aggrecan and collagen type II).

The incorporation of cell adhesion peptide into artificial ECMs may favorably influence stem cell behavior. Artificial ECM comprising a semi-interpenetrating polymer network, or polymer hydrogel, was designed to mimic the native ECM in terms of mechanical properties and cell adhesion properties. Poly(N-isopropylacrylamide-co-acrylic acid) |p(NIPAAm-co-AAc)| was loosely cross-linked with an acrylated peptide, Gln-Pro-Gln-Gly-Leu-Ala-Lys-NH2 (QPGQLAK-NH2), the sequence of which was designed to be cleaved by matrix metalloproteinase-13 and other collagenases [196]. The mechanical properties of the p(NIPAAm-co-AAc) hydrogel were controlled by varying the density of the network cross-linker. To provide cell adhesion properties to the hydrogel, the hydrogel was further interpenetrated by polyacrylic acid-graft-Ac-CGGNEPRGDTYRAY-NH2 |p(AA)-g-RGD| linear polymer chains, which provide an active RGD motif [197]. Human ESCs cultured in this hydrogel showed increased adherence and viability, as well as the expression of markers of undifferentiated human ESCs. In contrast, human ESCs cultured on gelatin-adsorbed polystyrene exhibited morphologies of spontaneously differentiating cells.

In addition to cell adhesion peptide conjugation, cell adhesion proteins such as fibronectin have been incorporated into artificial ECMs made of synthetic polymers. The surfaces of photo-crosslinkable PEG diacrylate scaffolds were modified by covalently conjugating fibronectin for the promotion of cell adhesion properties [198]. Mouse MSCs cultured on the fibronectin-conjugated PEG diacrylate hydrogel also adhered to the hydrogel and underwent osteogenic differentiation, as indicated by matrix mineralization [198]. Compared to free fibronectin, fibronectin conjugated to artificial ECM promotes cell proliferation [199]. On the other hand, conjugation of fibronectin to polyethylene terephthalate scaffolds results in higher proliferation of human umbilical cord blood CD34+ cells compared to free, soluble fibronectin. This result could have been due to the prevention of fibronectin detachment from the scaffolds by covalent conjugation, compared to the high frequency of fibronectin desorption that occurs with free, soluble fibronectin.

6.3. Chemistry of artificial ECMs

Hyaluronic acid is a polysaccharide found in many tissues and is known to influence stem cells’ behavior. Hyaluronic acid has been shown to induce chondrogenic differentiation of MSCs. Entrapment of hyaluronic acid in photo-crosslinkable PEG diacrylate hydrogel containing cultured MSCs enhances cartilage-specific gene expression [200]. In addition, MSCs cultured in hyaluronic acid hydrogels express a variety of ligament proteins [201]. Hyaluronic acid also influences ESC behavior; human ESCs encapsulated in hyaluronic acid hydrogels maintain their pluripotency and undifferentiated state, whereas human ESCs encapsulated in hydrogels of dextran, a similarly structured polysaccharide, undergo spontaneous differentiation [202]. Furthermore, simple addition of soluble growth factors to these hydrogels triggers lineage-specific differentiation of the entrapped ESCs.

Collagen was also shown to favorably modulate stem cells’ behavior. Human MSCs were mixed with collagen type II isolated from bovine cartilage and then cultured in pellet form [203]. The cells on the collagen type II scaffold underwent chondrogenic differentiation into cartilage-specific ECMs. Chondrogenesis of MSCs is most likely mediated by interaction with collagen type II through integrin β1, since blocking of this integrin impeded chondrogenesis. Implantation of type II collagen-cell constructs resulted in maintenance of chondrogenesis, inhibition of endochondral ossification and prevention of vessel invasion in vivo.

Hydroxyapatite can promote bone formation by stem cells. Hydroxyapatite is known to enhance osteoblastic differentiation as well as osteoblast growth [204]. A study previously demonstrated that PLGA scaffolds incorporating apatite on their surfaces can stimulate the regeneration of bone tissue in vivo by osteogenic cells derived from human ESCs [205]. Efficient apatite incorporation into PLGA scaffolds can be achieved through either gas foaming of PLGA-hydroxyapatite scaffolds [206] or biomimetic apatite-coating [207]. PLGA-hydroxyapatite composite scaffold exposed to high amounts of hydroxyapatite can be fabricated by the gas-forming or particulate-leaching method without the use of organic solvents. The surface of the PLGA-hydroxyapatite composite scaffold can further be coated with bone-like apatite by incubation in simulated body fluid [207]. The biomimetic apatite-coating process was accelerated by the introduction of
hydroxyapatite nanoparticle nucleation sites to the PLGA-hydroxyapatite composite scaffold. Bone-like apatite on the scaffold surface significantly enhances the in vivo osteogenic potential of the scaffold due to improved osteoconductivity [207]. Apatite-coated PLGA films enhance proliferation, alkaline phosphatase activity and osteocalcin production in human MSCs [208]. Further, the inclusion of hydroxyapatite particles in self-assembling peptide hydrogel promotes osteogenic differentiation of mouse ESCs [209].

6.4. Architecture of artificial ECMs

The macroscale, microscale and nanoscale structures of artificial ECMs can determine the fate of stem cells. The macroscale and microscale ECM structures affect cell migration, nutrient and waste diffusion and bulk mechanical properties. Generally, artificial ECMs are macroscopically designed to have physical properties (mechanical properties, pore size, porosity, and degradation rate) for the regeneration of specific tissues or organs. The resultant artificial ECMs may lack the nanoscale features required for precise control of cell behavior and tissue formation. Therefore, the nanoscale design of artificial ECMs is critical, since the nanoscale structure of the artificial ECMs can provide a substantial cellular interface for effective interactions with cells [210] as well as the modulation of cell behavior, including cell adhesion, biophysical force transmission, cell shape, cytoskeletal structure and differentiation [211,212].

Stem cells interact typically with nanoscaled ECMs in vivo, and this interaction may influence stem cell behaviors. Natural ECMs are composed of a complex network of ECM molecules with nanoscale and microscale dimensions, such as a nanofibrillar collagen fibril network. To investigate whether or not the nanostructure of the artificial ECMs influences stem cells’ behavior, 3D nanofibrillar, artificial ECMs were produced by depositing electrospun nanofibers composed of polyamide onto the surfaces of glass coverslips [213]. Culture on the nanofibrillar matrix greatly enhanced the proliferation and self-renewal of mouse ESCs compared to culture without nanofibers [213]. The dimensions of the nanotubular matrix significantly influence stem cell behavior. Human MSCs were cultured on nanotubular-shaped titanium oxide matrices, and osteogenic differentiation of human MSCs was induced by controlling the diameter of the nanotubes in the absence of osteogenic soluble factors [214]. As a result, nanotubes with large diameters (70–100 nm) were found to promote cell elongation and osteogenic differentiation of MSCs, whereas nanotubes with small diameters (30 nm) elicit cell adhesion only without noticeable differentiation.

Artificial nanofiber ECMs can be functionalized using bioactive factors to enhance stem cell differentiation. The addition of hydroxyapatite particles into self-assembling peptide nanofiber matrices promotes osteogenic differentiation of cultured mouse ESCs [214]. Further, incorporation of specific peptide sequences into self-assembling peptide nanofibers can promote neuronal differentiation of progenitor cells. Finally, incorporation of laminin epitope IKVAV into self-assembling peptide nanofibers promote neuronal differentiation of neural progenitor cells [215].

Stem cell differentiation is also significantly affected by the dimensions of the culture system, which may be due to differences in cellular interactions between cells cultured in 2D and 3D systems. In 2D culture systems, cells adhere to one side of the culture surface. In contrast, cells cultured in 3D hydrogels or scaffolds may attach to all sides of the hydrogels or scaffolds, to other cells, or even to secreted ECMs. ESC-derived EBs cultured in monolayer in the presence of chondrogenic soluble factors show limited expression of chondrogenic markers (aggrekan and Sox 9) [195,216]. However, when the EBs were cultured in 3D PEG hydrogels, the soluble factors dramatically enhanced chondrogenic marker expression. Therefore, a 3D culture environment more closely mimics chondrogenesis in vivo and may be responsible for enhanced chondrogenic differentiation.

Nanofabrication techniques have been used to study stem cells’ behavior in response to nanopatterned artificial ECMs. The nanopatterned surface of artificial ECMs can modulate diverse cell behaviors in terms of cell adhesion, cell orientation, cell migration, cytoskeletal change and gene expression [210]. Nanoscale topographical features that modulate cell behavior include scale (5 nm to micrometer scale), order type (e.g., ridge, step, groove, pillar and pit) and symmetry (e.g., orthogonal or hexagonal packing of nanopit) [217–223]. Increasing the nanoscale roughness of pore walls of artificial ECMs has been shown to enhance cell adhesion, proliferation and expression of ECM components [224]. Modulation of the nanoscale topography of cells may be mediated by changes in the adsorption and conformation of cell adhesion proteins, the type of integrin that binds to cell adhesion proteins and, consequently, integrin signaling [222]. Nanoscale topography may also modulate focal adhesion of the cells as well as cytoskeletal organization, both of which affect intracellular signaling [217].

6.5. Mechanical properties of artificial ECMs

The cellular microenvironments within tissues or organs can be physically diverse. The mechanical properties of the artificial ECMs on which cells reside can also vary as much as 300-fold from soft brain tissue to rigid, calcified bone [225]. Such variations in the mechanical properties of ECMs within various tissues are obvious during development [226,227], and such properties may play important roles in tissue or organ development [228]. Muscle cells prefer a compliant matrix for optimal contraction and relaxation, whereas bone cells prefer a rigid matrix on which they can mineralize. While the effects of growth factors on stem cell commitment have been well recognized, the importance of mechanical properties of matrices as a regulator of stem cell differentiation is just now being appreciated [229].

MSCs can sense the mechanical properties of an artificial ECM and transduce this information as changes in cytoskeletal organization or as stem cell commitment. Cells can feel the resistance to deformation of the artificial ECM by adhering to and pulling against the artificial
ECM. Physical forces are generated by cells adhered to the matrix due to the sliding of myosin bundles along actin filaments. Actin structures are linked to focal adhesions, which provide a pathway for the transmission of force from the cell to the matrix inside [230]. The tension generated from the cell-matrix interaction induces changes in cytoskeletal organization [231] as well as cellular contraction [232]. The biophysical cues are then converted into biochemical signals via signaling molecules that act as mechanotransducers [233]. Finally, the biochemical signals commit the cell to a specific lineage [229].

The mechanical properties of a matrix or culture surface can modulate stem cell differentiation. Engler et al. found that matrix elasticity directs the differentiation of human MSCs into cells the tissue type of which matches the stiffness of the culture environment [229]. Specifically, matrix elasticity was varied by controlling the cross-linking of polyacrylamide gels [234]. To promote cell-adhesive properties, the gels were coated with collagen type I, which is known to support both myogenic and osteogenic differentiation [235]. MSC differentiation correlated with the in vivo matrix elasticity. Soft matrices that mimic brain induced neurogenic differentiation, stiffer matrices that mimic muscle induced myogenic differentiation, and comparatively rigid matrices that mimic bone induced osteogenic differentiation (Fig. 7). Various mechanical signals were generated by the cells pulling on the matrices with various levels of elasticity. Moreover, the inhibition of nonmuscle myosin II, a cytoskeletal motor, blocks the modulation of cell differentiation by variable matrix elasticity.

The relationship between MSC differentiation and matrix elasticity in 2D culture systems [229] has also been demonstrated in 3D culture systems [236]. 2D culture systems for the modulation of stem cell differentiation may be incompatible with 3D tissue fabrication methods that use stem cells. Pek et al. demonstrated that mechanical properties modulate the differentiation of MSCs cultured in 3D gels [236]. They previously developed a thixotropic PEG-silica nanocomposite gel for 3D cell culturing [237]. This gel was used as a 3D matrix to investigate the effects of mechanical signaling on MSC differentiation in a 3D matrix. The stiffness of the gel was varied by controlling the amount of fumed silica [238]. When human MSCs were cultured in the gels with varying degrees of mechanical stiffness, the highest expression levels of neural (ENO2), myogenic (MYOG) and osteogenic (Runx2, OC) transcription factors were obtained using gels with low, medium and high degrees of stiffness, respectively [236]. In addition, the immobilization of RGD ligand to the gel further enhanced osteogenic factor expression, due possibly to the RGD ligand-integrin complex acting as a mechanical signal receptor that allows the transmission of signals through a signaling pathway [239].

6.6. Control of cell shape and size with artificial ECMs

Soft lithography techniques allow for spatial patterning of cell adhesion molecules, which can be utilized to control cell size and shape. Chen and colleagues showed that cell shape and size direct stem cell fate [240]. Micropatterned substrates containing fibronectin-coated islands of various sizes were fabricated using polydimethylsiloxane (PDMS) stamps. Human MSCs cultured on larger islands tended to flatten, spread and then differentiate into osteoblasts, whereas cells cultured on smaller islands tended to be round and unspread and differentiate into adipocytes. The shape-dependent control of differentiation was mediated by RhoA activity and cytoskeletal tension. Surprisingly, controlling RhoA activity did not require the use of soluble differentiation factors for adipogenic or osteogenic differentiation.

Cell shape also modulates differentiation of embryonic mesenchymal cells [241]. The role of embryonic mesenchymal cell shape in smooth muscle differentiation has been investigated by plating undifferentiated embryonic mesenchymal cells on microsurfaces. Microsurfaces with diameters of 10 and 20 μm were prepared by coating multi-perforated polycarbonate membranes with holes of identical diameter using tissue adhesive. Embryonic MSCs cultured on 10 μm diameter microsurfaces conserved their original round cell shape, whereas cells cultured on 20 μm diameter microsurfaces were elongated. The round-shaped cells remained undifferentiated and expressed α-fetoprotein, which is produced by fetal cells but not by differentiated cells. In comparison, the elongated cells differentiated into smooth muscle, as indicated by the expression of smooth muscle-specific proteins and membrane potential-dependent calcium ion currents. The cell shape was the critical factor in directing differentiation into smooth muscle, regardless of the supplementation of TGF-β1.

When cells are of identical size, cell shape becomes significant in modulating stem cell differentiation. A study by Chen et al. demonstrated that cell shape and size direct stem cell fate [240] and that cell shape also affects cell size. However, their study did not isolate the effect of either cell size or shape from each other. Kilian et al. investigated the effect of cell shape only on stem cell differentiation by varying cell shape and keeping cell size constant [242]. A microcontact printing technique using PDMS stamps was used to produce a patterned culture of human MSCs with various cell shapes and with identical cell sizes in the presence of both adipogenic and osteogenic soluble factors. Cells that were flower-shaped differentiated into adipocytes, whereas star-shaped cells resulted in osteogenic differentiation, possibly due to a difference in cytoskeletal contractility. A previous study showed that contractile cytoskeletal movement changes the cell from a flower shape to a star shape [243]. The concave regions between the points of the star are highly contractile regions where the cell spans across a non-adhesive area [242]. Star-shaped cells have larger focal adhesion and cytoskeletal tension than cells that are flower-shaped. It has been suggested that local curvatures that increase the cytoskeletal tension of the cell also promote osteogenic differentiation rather than adipogenic differentiation [242]. It was also suggested that cells with enhanced contractility undergo osteogenic differentiation due to the increased activation of c-Jun N-terminal kinase (JNK) and extracellular related kinase (ERK1/2) in conjunction with elevated wingless type (Wnt) signaling.
6.7. Mechanical signal loading on artificial ECMs

Stem cell differentiation into certain types of cells is influenced by the mechanical microenvironment. For example, cells in tissues or organs in vivo are subjected to various forms of mechanical forces. Cells of the heart, arteries, bladder and intestines are subjected to cyclic strain. Further, chondrocytes are subjected to cyclic compression in vivo. It is thought that the same forces that govern tissue development in vivo also enhance tissue development.
in vitro [244]. Thus, the mechanical signals could play significant roles in the lineage commitment of stem cells. For example, shear force [245] or cyclic strain [246] induces vascular cell differentiation of ESCs. It is speculated that mechanical signals are transmitted to the cells via cell surface receptors at focal adhesion sites, which serve as mechanical transducers between the cell-adhesion surface and cytoskeleton of the cell. The transmitted mechanical signals are converted into biochemical responses through a mechanism termed as mechanotransduction [247], resulting in activation of specific genes that modulate stem cell differentiation.

Mechanical stimulation alone, without exogenous growth factors, can induce the differentiation of bone marrow mesenchymal progenitor cells into a ligament cell lineage [248]. Collagen gel was used as an artificial ECM for the transduction of mechanical signals to cells in vitro. The application of ligament-like multidimensional mechanical strains (translational and rotational strain) to undifferentiated cells embedded in a collagen gel over a period of 21 days resulted in increased expression of ligament fibroblast markers, including collagen types I and III and tenasin-C, as well as induced cell alignment and collagen fiber orientation, all features characteristic of ligament cells.

Mechanical compression could promote the chondrogenic differentiation of stem cells. Hydrogels can be used as an artificial ECM for the transduction of compressive mechanical signals to cells. Exposure of bone marrow-derived MSCs cultured in PEG diacrylate hydrogels to cyclic compressive strain in vitro increases the gene expression of cartilage-related markers such as Sox-9, type II collagen and aggrecan regardless of TGF-β1 supplementation [112]. On the other hand, mechanical compression increases expression of cartilage-specific genes in human ESC-derived cells encapsulated in tyrosinearginine-glycine-aspartate-serine (YRGDS)-modified PEG-acrylate (YRGDS-PEG-acrylate) hydrogels only in the presence of TGF-β1 [112].

Elastic, artificial ECMs permit the transduction of mechanical cyclic strain signals for cardiomyogenesis. Cardiomyocytes in the body are subjected to cyclic strain induced by the heart beating. A study has demonstrated that cyclic strain promotes cardiomyogenesis of embryonic stem cell-derived cardiomyocytes (ESCCs) [249]. The application of cyclic strain to ESCCs cultured on elastic polymer [poly(lactide-co-caprolactone), PLCL] scaffolds in vitro elevated cardiac gene expression compared to unstrained controls. Elastic PLCL scaffolds were also used as a vehicle for cell transplantation into infarcted rat myocardium. PLCL scaffolds were seeded with ESCCs and implanted on the surface of infarcted rat heart. As the heart beats, cyclic strain signals were transmitted to ESCCs seeded on the elastic scaffolds. Importantly, cardiac gene expression was upregulated in the elastic patches compared to unstrained control patches (non-elastic PLGA scaffolds seeded with ESCCs), suggesting for the presence of cardiomyocyte-specific microstructures including myofibrillar bundles and Z-lines.

Mechanical signals in combination with soluble signals synergistically stimulate the differentiation of stem cells. Either cyclic strain [249] or TGF-β1 [250] alone has been shown to stimulate the cardiomyogenic differentiation of stem cells. Bhang et al. showed that combining a mechanical signal (cyclic strain) and soluble signal (TGF-β1) promotes cardiomyogenic marker expression in MSCs more effectively than soluble signal alone [251]. The expression of cardiac-specific markers (cardiac α-actin, tropomyosin, cardiac troponin-I and cardiac myosin heavy chain) was more upregulated in MSCs cultured with TGF-β1 and subjected to cyclic strain than in MSCs cultured statically with TGF-β1. Incomplete cardiomyogenic differentiation of MSCs could result in the persistence of undifferentiated stem cells that undergo unanticipated differentiation upon implantation. On the other hand, a combination of two or more types of signals could enhance the efficiency of stem cell differentiation.

7. Concluding remarks

Artificial ECMs can regulate the cellular function to engineer functional tissues. For engineering functional tissues, signals and parameters that can modulate cell behavior can be engineered into artificial ECMs. The signals and parameters include soluble signals, cell-adhesion signals, mechanical signals, and chemistry, architecture, and mechanical properties of artificial ECMs. Design criteria for artificial ECMs vary considerably depending on the tissue of interest. Artificial ECMs that can present appropriate signals and parameters can coordinate the interactions between the artificial ECMs and cells to regenerate a desired tissue. The artificial ECMs developed for tissue engineering may in turn provide a novel experimental system to elucidate the mechanisms by which native ECMs regulate tissue development and stem cell behavior.

Acknowledgements

This work was supported by a grant (ROA-2010-0018428) from NRL program of Korea Science and Engineering Foundation (KOSEF), the Ministry of Education, Science and Technology, Republic of Korea and a grant (2010-0020352) from the National Research Foundation of Korea.

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