Chapter 4
Engineering Biomaterials for Anchorage-Dependent and Non-anchorage-Dependent Therapeutic Cell Delivery in Translational Medicine

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4.1 Introduction

Translational medicine is an interdisciplinary field utilizing basic sciences and research findings to develop solutions to medical problems. Cell-based therapy, or utilizing biological cells as a medical intervention to replace lost or abnormal cells, tissues, and organs, has the potential to become an important therapeutic technology. Much research effort has thus been focused on developing cell delivery methods that can deliver and localize viable functional cells or undifferentiated stem cells to the target site with high efficiencies and at low cost. A multitude of biomaterials have been developed to act as contained cell carriers—confining cells within a scaffold structure to facilitate easy handling as well as prevent leakage or migration of cells from target site after implantation. This not only delivers cells into the target site, but it also creates a distinct separation between the host tissue and delivered cells, hence protecting against immune responses [1–4]. As different cells have different cell–cell and cell–material interactions, it is critical that biomaterials and their scaffolding structure are designed to suit the therapeutic cell type.

Ideally, materials used for cell delivery should be easy to fabricate at a low cost, be biocompatible, and biodegradable with no undesirable degradation products and with degradation rates matching those of tissue regeneration. They should also have suitable mechanical integrity for ease of handling. Furthermore, suitable biochemical and physical moieties that mimic the microenvironment niche of the cells to be delivered should ideally be present. The choice of appropriate biomaterials and their structure is therefore crucial for successful cell delivery [5]. In light of the complexity of these criteria for materials to ensure proper and efficient delivery of therapeutic cells, this chapter shall focus on the advancement

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of biomaterial research for the delivery of different cell types: anchorage-dependent cells (ADCs) and non-anchorage-dependent cells (non-ADCs). Furthermore, several key improvisations that are envisaged to propel cell delivery as a viable medical solution are discussed.

4.1.1 Anchorage-Dependent Versus Non-anchorage-Dependent Cells

Therapeutic biological cells can be broadly categorized into two types: anchorage dependent or non-anchorage dependent. ADCs in their native environment require extensive adhesion to the extracellular matrix (ECM) and exhibit a stretched or spreading morphology. ADCs include osteoblasts, fibroblasts, neural, epithelial, endothelial, and smooth muscle cells [6, 7]. On the other hand, non-ADCs exhibit a rounded morphology in native environment and do not require extensive cell adhesion to the surrounding ECM. These cells include blood cells, chondrocytes, hepatocytes, embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs).

ADCs' survival depends on a homeostatic mechanism whereby a specific apoptotic pathway is activated when the cell is unable to adhere to the surrounding ECM [6]. This apoptotic process, coined *anoikis* meaning "homelessness" in Greek, functions to prevent detached cells from being able to survive and proliferate dysplastically in non-native locations, which is especially important for preventing tumorigenesis [6]. This attachment-dependent survival has been widely documented in various cell types, including epithelial and endothelial cells.

In *anoikis*, cells that are not attached to their native ECM sense the loss of integrin (comprising α- and β-subunits) contact and translate these mechanical cues into intracellular signaling cascades, leading to apoptosis (Fig. 4.1) [8, 9]. There are namely two pathways—intrinsic and extrinsic—that both ultimately effect a caspase cascade, leading to endonuclease activation and DNA fragmentation [10]. When cells are properly attached to their appropriate ECM, anti-apoptotic signals Bcl-2 and Bcl-XL are expressed to maintain mitochondrial membrane's integrity by binding to pro-apoptotic Bad and Bax and sequestering pro-apoptotic Bim [11, 12]. In the intrinsic pathway, when cells fail to attach to the ECM, integrin disengagement increases the amount of Bim in the cytoplasm by allowing its release from cytoskeleton while preventing its degradation (inhibition of ERK and PI3K/Akt-mediated phosphorylation of Bim) [10]. Apoptosis activators Bim and Bid as well as apoptosis sensitizers Bad, Puma, Bik, Noxa, Hrk, and Bmf are activated [10, 13]. Apoptosis sensitizers on the cell membrane act as competitive inhibitors of apoptosis activators for Bcl-2 [14, 15]. Apoptosis activators, which are usually repressed by anti-apoptotic Bcl-2 and Bcl-XL, then assemble Bax and Bak into Bax–Bak oligomers in the outer mitochondrial membrane (OMM) [16]. The OMM is hence permeabilized, and cytochrome c is

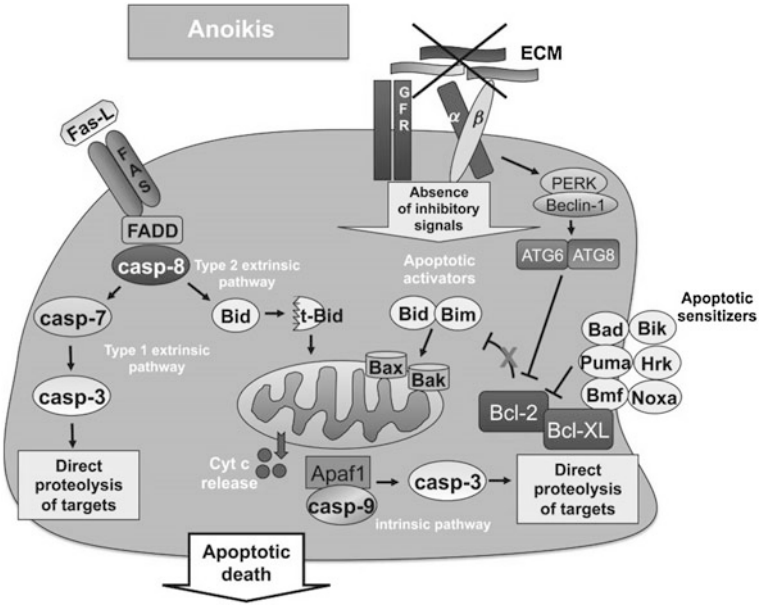

Fig. 4.1 *Anoikis* activation pathways. Lack of adhesion to the extracellular matrix via integrins (transmembrane proteins consisting of α- and β-subunits) reduces the anti-apoptotic signals Bcl-2 and Bcl-XL. The pro-apoptotic pathways involving increase in pro-apoptotic molecules Bid, Bim, Bad, Puma, and Noxa are therefore not inhibited, leading to an intracellular caspase cascade that causes cytochrome c (cyt-c) in mitochondria to be released and apoptosome assembly in the intrinsic pathway. The extrinsic pathways are activated by extracellular death ligands such as FasL binding to corresponding death receptors Fas. There are two branches of the extrinsic pathway: The first leads to a caspase cascade resulting in the direct proteolysis of target proteins, while the second results in a truncated Bid (t-Bid) that joins the intrinsic pathway to activate cytochrome release from mitochondria. [9] Recreated from Fig. 1 of Ref. [9] with kind permission from John Wiley and Sons (License Number 3125710475964)

released from the mitochondria into the cytoplasm [9]. Subsequently, cytochrome c binds with caspase-9 and apoptosis protease-activating factor (APAF) to form an apoptosome which activates caspase-3, resulting in a caspase signaling cascade concluding in apoptosis [17, 18].

There are two branches of the extrinsic pathway, both of which begin with the binding of extracellular death ligands Fas ligands (FasL) and tumor necrosis factor-α (TNF-α) to the cells' corresponding transmembrane receptors Fas and TNF-α receptor TNFR, which are upregulated upon disengagement from ECM [19, 20]. The morphological change into a rounded cell further causes the accumulation and activation of the mentioned receptors [21, 22]. FasL binds to its receptor present on the cellular membrane, causing death-inducing signaling complex (DISC) to be formed. Subsequently, DISC causes the activation of caspase-8 that activates a caspase cascade involving caspase-3 and caspase-7, causing the proteolysis and ultimately cell death (type I extrinsic apoptosis); caspase-8 also

can cleave pro-apoptotic Bid into a form (t-Bid) that encourages apoptosome assembly and cytochrome c release, facilitating cell death in the second type of apoptosis [9].

4.1.2 Biomaterials in Cell Delivery Vehicles

A wide variety of biomaterials, both naturally derived and synthetic, have been used in constructing three-dimensional (3D) cell delivery vehicles or cell-laden scaffolds. These scaffolding systems have to fulfill several basic criteria in order to successfully encapsulate cells in vitro and subsequently deliver the functional cells to the target site in the clinical setting. Most importantly, biomaterials used must be biocompatible, that is, they must possess the ability to perform in vivo without invoking any harmful effects such as immune response [23]. Secondly, they should provide a suitable microenvironment for cells to attach, reside, and proliferate within. Thirdly, biomaterials should be biodegradable with a controlled rate—degradation by-products should not be harmful to cells and the rate should match that of tissue growth. Lastly, as cell delivery vehicles, suitable mechanical integrity for ease of handling ex vivo and similar mechanical properties as surrounding tissues in vivo is critical. Simply put, the cell delivery vehicle should recapitulate both the micro- and macroenvironments of the target implantation site; therefore, the materials utilized in constructing the vehicle are of critical importance.

Biomaterials can either be naturally derived or be synthetically manufactured (Table 4.1), both of which possess respective pros and cons. Naturally derived biomaterials are popular because of their low toxicity, biodegradability, low cost of manufacture, and possession of cell-adhesive moieties. However, it is precisely this ability to interact with cells that also imparts the risk of immune response and disease transfer; strict screening and purification are required. Batch-to-batch variation and therefore uncontrollable mechanical properties and degradation rates are also cause for concern.

Synthetic biomaterials circumvent the need for purification and batch-to-batch variation. Synthetic polymers have predictable and controllable chemical and physical properties, but are more expensive and do not possess cell adhesion moieties. Furthermore, their degradation by-products may be toxic to cells. Therefore, synthetic materials require functionalization to be biocompatible. Popular synthetic biomaterials include poly(ethyl glycol) (PEG), polylactic acid (PLA), and poly(vinyl alcohol) (PVA). PEG is not naturally degradable and cell adhesive, requiring addition of cleavable segments such as polyester units and coatings with cell adhesion moieties.

Often, the best of both worlds—respective advantages of naturally derived and synthetic biomaterials—are combined by researchers in a bid to create an “ideal” biomaterial with the desired properties for the target delivery cell type and the target implantation site [24].

Table 4.1 List of popularly used naturally derived and synthetic biomaterials for cell delivery

Naturally derived	Synthetic
Alginate	Poly(acryl amide) (PAam)
Agarose	Poly(acrylic acid) (PAA)
Chitosan	Poly(ethyl glycol) (PEG)
Chondroitin sulfate	Poly(ethylene oxide) (PEO)
Collagen	Polylactic acid (PLA): poly-L-lactic acid (PLLA), poly-DL-lactic acid (PDLLA)
Elastin	Poly(lactide-co-glycolide) (PLGA)
Fibrin	
Gelatin	
Hydroxyapatite	Poly(vinyl alcohol) (PVA)
Hyaluronic acid	Poly(ε-caprolactone) (PCL)
Silk fibroin	

4.2 Engineering Basic Cell Delivery Structures

Cell delivery vehicles (or scaffolds) can be categorized into preformed or injectable systems. Preformed systems are fabricated ex vivo; cells are introduced either during or after the fabrication process of a macroscopic-sized scaffold. The cell-laden scaffolds are then cultured in vitro for a period of time prior to surgical implantation. On the other hand, injectable systems are either in cell-encapsulated microsized scaffold (microcarrier) form or in a cell suspension form, which can be easily injected into the target site, thereby providing a minimally invasive means of implantation. The latter refers to hydrogel solutions that can be gelled in situ under mild conditions, imparting the quality of filling up irregular defects.

In the subsections below, the different fundamental structures and their fabrication techniques, as well as the popular biomaterials used in cell delivery vehicles are discussed. Biomaterials are often engineered to achieve quick and simple polymerization as well as to suit the cell type and the target site; they can be conjugated with polymerizable segments, blended or copolymerized with other materials prior to fabricating the scaffold structures or fabricated with certain orientations. Table 4.2 summarizes and compares the four different basic structures.

4.2.1 Hydrogels

Hydrogels are a class of insoluble water-swollen polymeric networks formed from crosslinked water-soluble macromers. Usually, cells are mixed into the hydrogel precursor solution prior to gelation, hence achieving a homogeneous cell-laden hydrogel (Fig. 4.2a). Advantages of hydrogels are as follows: the highly hydrated environment and good diffusion of nutrients and waste as well as the mimicking of

Table 4.2 Features, fabrication techniques, and development of basic cell delivery structures

	Hydrogel	Fibrous mesh	Sponge	Decellularized scaffold
Features ✔: advantages - : disadvantages	✔ Highly hydrated, mimicking biological tissue	✔ Fibers mimic ECM fiber architecture in structural tissues (collagen)	✔ Highly porous ✔ Strong mechanical properties, suitable for structural tissues	✔ Provides tissue-specific native ECM and growth factors (niche) by previous residing cells
	✔ Good diffusion of nutrients and waste ✔ Mild conditions for polymerization (fabrication) ✔ Reversible gelation (responsive to external stimuli, e.g., change in temperature and ionic concentration) ✔ Typically degradable ✔ Fast and simple gelation ✔ Injectable – Weak mechanical properties, suitable for soft tissues only	✔ Strong mechanical properties, suitable for structural tissues, e.g., bones ✔ Highly controllable physical and chemical parameters ✔ Controllable fiber diameters (nm to μm scale) – Time-consuming, labor intensive – Non-injectable – Require prefabrication before cell seeding	– Small, thin templates for proper diffusion – Limited amount of pores for structural integrity; limited interconnectivity – Irregular pore size – Difficulty in ensuring well-distributed pores – Require removal of toxic components used in fabrication – Time-consuming, labor intensive post-fabrication washing – Require prefabrication before cell seeding	✔ Able to retain complex structure and ECM architecture that cannot be easily mimicked (e.g., alveoli of lungs) ✔ Biocompatible ✔ Xenogeneic and allogeneic sources used without eliciting immune response as ECM components are well conserved among species – Labor intensive, time-consuming as complete removal of cells and nuclear material required – Decellularization protocols may cause structural ECM changes
Fabrication techniques	• Photoinitiated (conjugate with acrylate groups) • Enzymatic (conjugate with phenolic hydroxyl groups) • Temperature (e.g., agarose, gelatin) • pH (e.g., chitosan) • Ionic (e.g., alginate) • Self-assembly peptides/ amphiphiles	• Electrospinning • Extrusion • Phase separation	• Gas foaming • Particulate leaching/ solvent casting • Freeze-drying • Phase separation • Thermally induced phase separation (TIPS)	• Physical (freeze–thaw, agitation, sonication) • Enzymatic (trypsin, endonucleases) • Chemical (SDS, Triton X-100)

(continued)

Table 4.2 (continued)

	Hydrogel	Fibrous mesh	Sponge	Decellularized scaffold
Modification	• Interpenetrating network (IPN) of two hydrogels [27] • Saccharide–peptide hydrogels [43, 44]	• Blending of materials [122, 123] • Coatings [56] • Aligned fibers [58] • Different shapes, e.g., tubes and mats • Zonal organization [55]	• Blending of materials [62, 124]	• Conversion into hydrogel [82] and sponge [79]

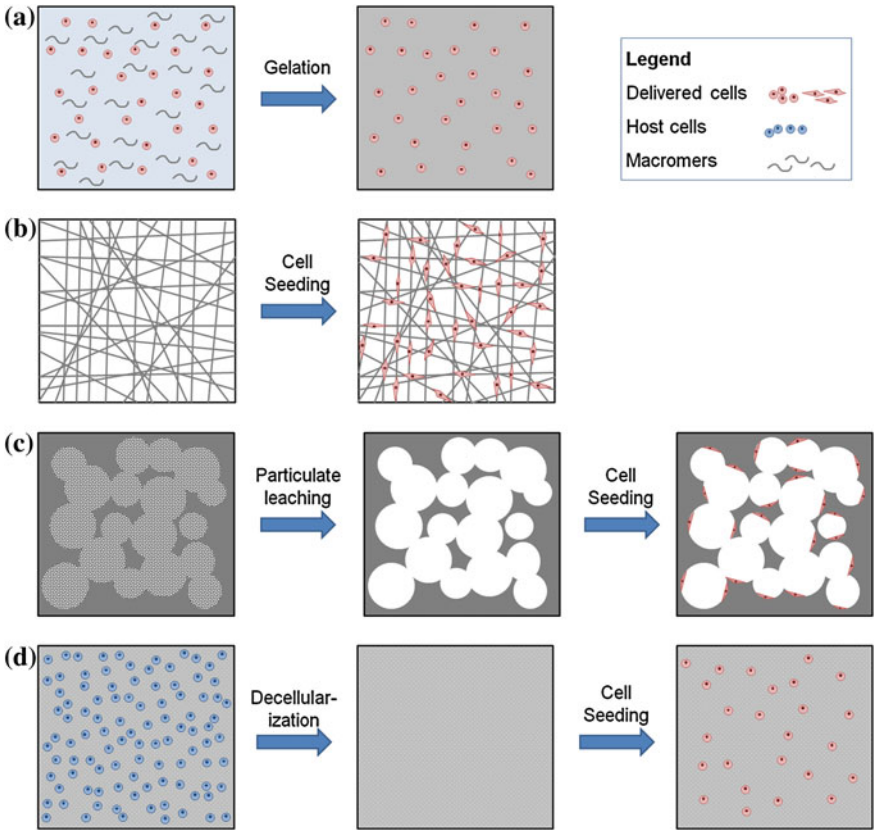

Fig. 4.2 Basic cell delivery structures. (**a**) Hydrogel formation from homogenous cell suspension in macromer solution; (**b**) fibrous mesh fabrication via electrospinning and subsequent cell seeding and attachment onto fibers; (**c**) sponge fabrication via particulate leaching by firstly forming a solid encapsulating particulates (porogens), leaching of particulates to leave behind pores, and finally seeding cells that attach to walls of pores; and (**d**) decellularized scaffolds fabrication by firstly isolating biological tissues and organs from allogeneic or xenogeneic sources, applying decellularization techniques to remove previous host cells without breaking down ECM structure and finally seeding therapeutic cells onto decellularized ECM scaffold

most tissues. Many hydrogels offer the advantage of being injectable: In other words, they can be quickly gelled in situ under mild physiological conditions, thereby allowing molding into irregularly sized defects via a minimally invasive means of implantation. These properties have thus made hydrogels popular for cell and drug delivery. Through altering the porosity and crosslinking density, mechanical properties of hydrogels can be adjusted to suit the targeted cell type and site of implantation. Mechanical strength, however, is usually low compared to the meshes and sponges, and hence, hydrogels are only suitable for soft tissues such as cartilage. Furthermore, as hydrogels are highly hydrophilic, cells typically

exhibit a rounded morphology when encapsulated within [25]. While this makes hydrogels suitable for the delivery of non-ADCs, successful delivery of ADCs using hydrogels would require modifications to include cell adhesion moieties within the hydrogels for ADCs to adhere onto.

Hydrogel formation (gelation) is attained via physical or chemical crosslinking mechanisms. Under suitable conditions, a solution consisting of macromers or unreacted monomers with crosslinking agents is converted into an insoluble 3D network upon gelation. Physically crosslinked gels are linked via ionic or hydrogen bonds or hydrophobic interactions, forming a gel upon a change in environmental conditions, e.g., temperature, pH, and ionic concentration. Temperature-responsive hydrogels such as agarose and gelatin have reversible properties. Alginate, derived from brown algae, is an ionic polysaccharide that is crosslinked upon presence of divalent cations such as calcium. These are usually reversible crosslinking reactions—upon reversal of conditions, the gel reverts to a solution form. Chemically crosslinked hydrogels are linked via covalent bonds—radical initiators activate crosslinking agents that link monomers to a certain critical density that converts the precursor solution form into a gel form. An example would be Photoinitiated polymerization using ultraviolet or visible light, which is a popular crosslinking technology because it offers injectability [26].

Photoinitiated crosslinking is a popular technique using ultraviolet (UV) radiation, whereby gelation is achieved at physiological temperature and pH under light exposure. Macromers such as PEG are firstly conjugated with acrylate groups and subsequently mixed with a small amount of photoinitiators and cells prior to exposure to light for gelling. Depending on the mechanical strength required which is dependent on crosslinking density, the density of conjugated acrylate groups or the duration of light exposure can be varied. Alginate was conjugated with methacrylate through an esterification reaction to become photoinitiated crosslinkable [27]. To provide the cell adhesion moieties, the alginate–methacrylate precursor was mixed with temperature-responsive collagen solution and gelled by firstly increasing the temperature to 37 °C for collagen gelling and then exposing to UV light for alginate crosslinking, thereby fabricating an interpenetrating network (IPN) of two different hydrogels. This IPN hydrogel was reported to have a denser network and hence had superior mechanical properties than photoinitiated crosslinked alginate gel (control). Furthermore, it was able to support murine preosteoblasts MC3T3-E1 (as an ADC type) for bone defect repair in terms of extensive cell spreading morphology, high proliferative rates, and maintenance of osteogenic gene expressions as compared to the control.

Enzymatically crosslinked gels have recently been gaining popularity due to its injectability and gelation under physiological conditions. A gel can be formed in situ by mixing the phenolic hydroxyl-conjugated macromer solution with hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP) enzyme in the target defect site. This peroxidase-catalyzed system has been applied in various polysaccharides including chitosan, hyaluronic acid, alginate, and dextran [28–32]. Notably, gelatin type A was modified to possess tyramine (phenolic hydroxyl

groups), which was subsequently used for the delivery of osteoblasts [33]. Murine preosteoblasts MC3T3 cells were mixed in the tyramine-conjugated gelatin solution and gelled upon the addition of HRP. The osteoblasts were observed to have a spreading morphology even though osteogenic gene and protein analyses were not significant. Inclusion of bioactive molecules that can promote the osteogenic phenotype, e.g., bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), may be beneficial [34, 35]. Recently, Mathieu et al. injected a suspension of mesenchymal stem cells (MSCs) and a pH-responsive hydrogel into infarcted myocardia of rats and observed an increased healing rate and functionality with little fibrosis [36]. The silane-grafted hydroxypropyl methylcellulose (Si-HPMC) gels upon a decrease in pH levels to the physiological pH 7–7.4, due to the condensation of silane groups.

Self-assembling peptides spontaneously assemble themselves into a stable macroscopic nanofibrous network via non-covalent interactions such as hydrogen bonding, hydrophobic and Van der Waals forces [37]. Being amphiphilic, i.e., containing both hydrophilic (polar) and hydrophobic (non-polar) amino acid residues, the peptides undergo self-assembly to undertake the most stable structure as a nanofiber in a polar environment (salt solution or cell culture media)—non-polar residues are orientated into the fiber center while exposing the polar residues to the environment. This phenomenon is ubiquitous in nature, e.g., phospholipids in forming micelles and plasma membranes. As the self-assembled scaffolds are simple and form under physiological conditions, have a fiber diameter of approximately 10 nm and pore diameter of 5–200 nm and mimic ECM structure, are highly hydrated, and can be functionalized with the addition of cell adhesion moieties, they have been extensively utilized as a tissue engineering scaffold [37, 38].

For example, to impart the cell adhesion capability to the self-assembled nanofibrous hydrogel, cell adhesion moieties such as arginine–glycine–aspartic acid (RGD) sequences can be conjugated to the peptides prior to assembly [39–41]. Zhou et al. [39] designed a self-assembled hydrogel based on two simple peptides, one of which possesses the RGD sequence. The sequence not only provides the hydrogel with cell-adhesive properties on the surface of the nanofibers, but it also influences the mechanical properties of the hydrogel. Human dermal fibroblasts encapsulated within the hydrogel were able to adhere through RGD-specific binding and exhibited a spreading morphology as ADCs do. Webber et al. [41] also demonstrated the potential of self-assembled hydrogels made up of RGD peptides in delivering bone marrow mononuclear cells (BMNCs) into mice. BMNCs were able to adhere and proliferate in the tested hydrogel in vitro, enhancing the cells' numbers by more than 5-folds in 5 days, whereas non-RGD-containing nanofibrous hydrogel control had no significant increase in cell number during the same time period. Furthermore, gene expression studies validated that BMNC phenotype was maintained in vitro; in vivo experiments also confirmed that BMNCs were successfully delivered subcutaneously with maintenance of high viability, albeit with a mild tissue reaction to the cell delivery material.

Gong et al. [42] made use of a naturally occurring phenomenon involving non-ADCs and the non-cell-adhesive property of hydrogel to develop a dense tissue construct for cartilage tissue engineering. A rapid and dense outgrowth of cells and ECM secretion at gel–medium boundaries, termed "edge flourish," was exploited in a microcavitary gel (MCG)—chondrocytes and gelatin microspheres were co-encapsulated in temperature-responsive agarose hydrogel; upon raising the temperature to 37 °C, gelatin melts to leave behind microcavities within the hydrogel bulk. The strategy was named phase transfer cell culture (PTCC) to connote the dynamic culture of cells on the boundaries of two phases. Chondrocytes were then observed to infiltrate and fill up the cavities. By inducing cavities to increase the amount of gel–medium boundaries throughout the hydrogel bulk, not only was diffusion of nutrients and waste improved, a higher proliferation rate and hyaline cartilage-specific ECM secretion was observed [42]. In another study involving a temperature-responsive hybrid hydrogel of naturally derived heparin and synthetic PEG diacrylate (PEGDA) was shown to support primary hepatocyte spheroids more greatly than pure PEG-based hydrogels, as concluded from the high cell viability and albumin and urea secretion [24]. The heparin–PEG hydrogel gels at 37 °C within a short duration of 10 min under physiological conditions and was also able to retain hepatocyte growth factor (HGF) and maintain its bioactivity; this hydrogel is therefore envisaged to be a potential stem cell delivery vehicle with differentiation cues incorporated within.

A relatively new synthetic saccharide–peptide hydrogel has been designed and demonstrated to be highly supportive of non-ADC chondrocytes [43] and pancreatic islets [44]. Being composed of naturally derived monomers saccharides and peptides, the intrinsic advantages of biodegradability, non-toxicity, and low cost are combined with the controllable and predictable properties of synthetic biomaterials. The copolymer backbone is covalently crosslinked via Michael-type addition mechanism and subsequently functionalized with tyrosine amino acids which, in chondrocyte studies, supported extensive ECM secretion and higher mechanical strength [43]. In another study involving pancreatic islet delivery into rat models using the same gel, the diabetic condition was significantly reversed alongside the high insulin secretion by the functional islets and lack of detectable immune response, as compared to transplanted unencapsulated islets [44].

4.2.2 Fibrous Meshes

Fibrous meshes are a body of individual nanoscale fibers that have been spun into a 3D network. Its structural similarity to native ECM network, high surface area to volume ratio, porosity, tunable mechanical and degradation properties, and controllable fiber diameter [45] makes fibrous meshes as suitable cell delivery vehicle, especially for ADCs (Fig. 4.2b). Electrospinning is a common technique for fabricating fibrous meshes. The droplet of polymer solution forms a thin stream upon passing through an electric field and is collected as a mesh of fibers upon the

evaporation of solvent in the polymer. By modifying the collector and electric field, fiber orientation [46] as well as scaffold shape and size [47–49] can be varied. For example, tubular scaffolds were fabricated using a rotating rod collector [50]; tubular structures with longitudinally aligned nanofibers were fabricated using two extra parallel electrodes [47]. However, the difficulty in cell penetration into a dense scaffold is a shortfall [51]. Naturally derived materials including elastin [52], sulfated silk fibroin [53], collagen [54], and synthetic materials PCL [46, 55] and PLA [56] have been used in fabricating electrospun scaffolds.

While many previous studies utilized simple randomly aligned fibrous meshes, second-generation fibrous meshes with aligned fibers and multilayered structures have been engineered to better mimic the complex native ECM structure. Since fiber orientation can modulate cell phenotype and guide cell growth, many studies have deliberately aligned fibers for the culture of muscle cells and neurons. For example, smooth muscle cells (SMCs) cultured on longitudinal poly(L-lactide-co-ε-caprolactone) P(LLA-CL) 75:25 fibers had a contractile phenotype similar to those in physiological conditions [57]. Another research group demonstrated the effect of aligned PCL/collagen fibrous meshes on skeletal muscle cells [58]. The skeletal muscle cells were stretched along the fibers, exhibited high viability and functionality with formation of myotubes observed.

In a bid to restore the neural retina through cell-based therapy, biodegradable PLA was utilized in fabricating a scaffold of radially aligned nanofibers mimicking the ECM architecture in the retina, for the culture of retinal ganglion cells (neurons) [56]. The scaffold was then immersed in laminin to coat the fibers as neurons reside in a laminin-rich ECM [59]. Axons of the cells, as a type of ADCs, were observed to be of higher viability and were mostly aligned along the fibers, as opposed to control tissue culture plates and randomly aligned electrospun scaffolds. Cells cultured in the aligned scaffolds maintained electrophysiological functionality and exhibited similar radial patterns as axon bundles of the native retina. Furthermore, at higher densities, the cells were observed to form axon bundles in vivo. This cell delivery vehicle for the treatment of degenerated retina, e.g., due to glaucoma, is an important first step in delivering aligned functional nerve cells and can be developed further for delivering other nerve cells, especially for the repair of the central nervous system, since nerve cells have poor self-repair capability.

Homogeneous fibrous meshes have been widely used as ECM substitutes, but recently the paradigm shifted toward creating structures more similar to native tissue. For example, the zonal organization of articular cartilage was mimicked using PCL fibers of varying orientations to match the mechanical properties of each zone: The topmost superficial layer as a lubricating surface was made of aligned 1-μm-thick fibers; the middle layer was composed of random 1-μm fibers; and the deep layer was composed of random and thicker fibers of 5 μm diameter, mimicking the architecture of collagen fibrils in native articular cartilage tissue [55]. Chondrocyte culture in the triple-layered electrospun scaffold was done in vitro for 5 weeks; analyses of mechanical properties and quality of engineered

cartilage revealed close similarity with native cartilage tissue. In another study, McClure et al. [60] designed a triple-layered electrospun scaffold with layer-specific compositions of PCL, elastin, and collagen fibers to mimic the structural architecture of arteries, namely the intima, media, and adventitia layers; the engineered construct was mechanically tested acellularly and demonstrated similar modulus and compliance values to those of native porcine femoral arteries. The potential of this artery-mimicking electrospun scaffold, however, would be better reflected with the culture of vascular cells—vascular endothelial cells and SMCs.

4.2.3 Sponges

Also known as porous scaffolds, sponges are made using gas foaming, particulate leaching, and freeze-drying techniques. The success of porous scaffolds as a cell delivery vehicle depends on its porosity, pore size, and pore interconnectivity—the pores provide surface area for cells to adhere on, while interconnectivity dictates the cell penetration as well as diffusion of nutrients and waste [25]. Pore size is critical: Small pores will impede cell penetration and proper diffusion, while larger pores may not be sensed by cells to be a 3D environment. Hence, engineering a cell delivery capable sponge requires consideration of pore design parameters.

Gas foaming involves the saturation of high-pressure gas (usually carbon dioxide) into the polymer particles; upon rapid decrease in pressure, gas bubbles nucleate and expand and the polymer fuses to form a continuous porous scaffold [61]. This method is quick and simple and allows the control of pore size by varying pressure without the use of high temperatures or harsh organic solvents to create the pores. Recently, PDLLA/PEG copolymer sponges were fabricated by gas foaming, achieving 84 % average porosity [62]. PDLLA possesses superior mechanical properties and biodegradability but is hydrophobic and therefore unsuitable for cell encapsulation. Hence, by copolymerizing with hydrophilic PEG, the advantageous properties of each material are combined. Although no cell-seeding studies were done on this sponge, this is a classic example of exploiting the advantages of two different materials by copolymerization, and with further optimization, the sponge is expected to work well as a cell delivery vehicle. Zhou et al. [63] combined foaming and blending of two immiscible polymers to create a more controllable, open, and porous scaffold with higher interconnectivity between pores. Firstly, two polymers PLA and polystyrene were blended in equal weight ratios together and molded by compression prior to carbon dioxide foaming, achieving a porous scaffold of PLA and polystyrene with tunable pore sizes. Then, polystyrene was removed via immersion of scaffold in cyclohexane, creating a PLA scaffold with much higher interconnectivity. After washing, osteoblasts were seeded onto the scaffold and compared against unfoamed control; although both scaffolds showed proper cell adhesion and spreading morphology of osteoblasts, the foamed sample had significantly higher number of cells, thereby reiterating the importance of porosity.

Particulate leaching using salt particles is an alternative method to create porous scaffolds (Fig. 4.2c) by mixing salt crystals (typically sodium chloride NaCl) into a polymer solution and then inducing the crosslinking to form a scaffold with salt particles entrapped within. Upon immersing in water, the salt dissolves and is removed from the scaffold, leaving behind empty spaces inside the scaffold. Sponges of silk fibroin/hyaluronic acid blends for cartilage tissue engineering were fabricated using this method [64]. Silk fibroin/PDLLA sponges were fabricated by NaCl particulate leaching; the silk fibroin was incorporated to increase the vascularization potential of the PDLLA sponge for bone tissue engineering, since silk fibroin is known to support the adhesion and growth of endothelial cells [65]. In this study, human umbilical vein endothelial cells (HUVECs) were shown to be properly adhered onto the silk fibroin/PDLLA sponges with high viability and proliferation while maintaining the cell phenotype in vitro; in vivo studies showed quicker vascularization and integration with the surrounding tissue.

PLA/PCL (70:30) sponges were also fabricated using NaCl particulate leaching and studied for the delivery of myoblasts for skeletal muscle repair [66]. Myoblasts were not only able to adhere and grow on the sponges, but they also differentiated and fused with each other into myotube structures with expression of skeletal muscle-specific genes and proteins, both in vitro and in vivo. Coupled with the biodegradability and biocompatibility of the materials, the PLA/PCL sponge exhibits great potential for skeletal muscle repair.

4.2.4 Decellularized Scaffolds

Biological tissues and organs comprised of ECM network and residing native cells can be extracted and then decellularized (removal of cells), leaving behind an intact ECM scaffold on which cells can be reseeded prior to implantation (Fig. 4.2d) [67]. Decellularized scaffolds provide a most suitable natural microenvironment as tissue- and organ-specific ECM cues produced and modeled by the previous residing cells can guide the newly seeded cells. Complete decellularization is important to remove the immunogenicity caused by previous cells' antigens, since the host tissue is of xenogeneic and allogeneic origin. This can be achieved by chemical (SDS, Triton X-100) [68], physical (freeze–thaw cycles), or enzymatic (trypsin, endonucleases) means [69]. Indeed, the excellent biocompatibility and lack of immunogenicity of decellularized scaffolds have been recognized and approved by FDA for bone [68], skin [70], and heart valve replacements [71] as acellular scaffolds, whereby host cells are allowed to penetrate and regrow tissue. Other decellularized scaffolds currently being studied and characterized include skin [72, 73], larynx [74], lung [75, 76], blood vessels [77, 78]. To accelerate the healing process, cells can be seeded within the decellularized scaffold and cultured in vitro, thereby generating a replacement tissue or organ ready for implantation [79, 80].

Wang et al. [80] characterized decellularized scaffolds from porcine musculofascial tissues and observed the presence of vascular endothelial growth factor (VEGF) in the decellularized ECM, which served to be pro-angiogenic and showed high healing capability when seeded with adipose stem cells (ASCs) into a rat muscle defect model. Lang et al. [81] optimized liver tissue decellularization and utilized decellularized liver tissue for studying the behavior of seeded human primary hepatocytes. The viability, phenotype, and functionality of the liver cells were highly conserved in a 3D environment for at least 21 days, which was significantly better than current collagen sandwich culture methods. In another study involving the complex lung tissue, Petersen et al. [76] decellularized rat lungs without disrupting the ECM architecture of airways, alveoli, and blood vessels, after which lung epithelial and endothelial cells were seeded onto the decellularized lung scaffold and subsequently tested both in vitro and in vivo. In vitro characterization showed maintenance of ECM components and lung structure, as well as seeded cells' phenotype. The engineered lungs were also able to function in vivo, as observed by the inflation of the organ, gas exchange, and hemoglobin saturation. Therefore, through the favorable results obtained in various studies, decellularized scaffolds of biological tissues and even whole organs were shown to be choice cell delivery methods in the form of whole-organ transplantation, if the abovementioned three engineered structures are unable to recreate complex architecture of tissues and organs.

However, decellularized tissues need not be used wholesale; instead, researchers have solubilized the ECM and converted them into porous scaffolds [79] and hydrogels [82–84]. In another study, Yu et al. [79] fabricated porous scaffolds from human decellularized adipose tissue: The decellularized tissue was solubilized by α-amylase, after which the solubilized proteins were made into porous scaffolds via freezing and lyophilization. The porous scaffolds were shown to support ASC adhesion and adipogenic differentiation. Wolf et al. [82] developed and characterized both in vitro and in vivo ECM-based hydrogels derived from decellularized dermal and urinary bladder tissues. The study validated that ECM and hence properties are tissue specific, therefore causing varied cell behaviors as well.

4.3 Improvising the Cell Delivery Systems

The basic cell delivery structures discussed above may still be inadequate on their own. As mentioned, research efforts based on combining different materials by copolymerization and blending to create a material with combined advantageous properties have been prevalent. Fabrication techniques are diverse and continuously improving to become milder, more elegant, and non-toxic. In this section, several key advancements that will potentially drive cell delivery toward the

clinical setting are noted. These include making the system injectable as an elegant and minimally invasive means of surgical implantation, functionalizing inert surfaces with bioactive ligands and molecules, controlling cell behavior using micro- and nanoscale topographical cues, as well as delivering intact masses of cells without delivering biomaterials. These promising developments not only make cell delivery vehicles simple systems, but they also can influence the delivered cells' behavior and phenotype.

4.3.1 Microcarriers for Injectability

Microcarriers are injectable cell or drug-eluting microscopic-sized vehicles that are downscaled versions of the abovementioned basic structures. Cell microcarriers can be further subcategorized into microsized scaffolds that ADCs can attach onto and microsized materials encapsulating cells within. The former are usually developed in two phases: The microcarriers are firstly fabricated, and the cells are subsequently seeded onto them. On the other hand, cell-encapsulating microcarriers are usually hydrogel based, using a one-step process of directly gelling microsized droplets of cell–material suspension. Several commercially available microcarriers such as Cultispher® (Sigma) have been used [85].

The emulsion technique, either a single or double emulsion, is a simple and therefore popular technique for microsphere fabrication. Huang et al. [86] fabricated gelatin type A porous microspheres using a single water-in-oil emulsion followed by freeze-drying; next, basic fibroblast growth factor (bFGF) was loaded into the rehydrated microspheres and finally fibroblasts were seeded. The typical spreading morphology of fibroblasts was exhibited on the microspheres, and implantation of the fibroblast/bFGF-loaded microspheres in critically sized dermal defects (which would require skin grafting) showed favorable healing—the epidermal layer was fully restored and was integrated with native tissue [86]. In another study, the same group utilized the same microsphere fabrication technique, but incorporated epidermal growth factor (EGF) instead of bFGF and mesenchymal stem cells (MSCs) instead of fibroblasts [87]. The MSC-laden microspheres were suspended in a type I collagen/Matrigel™ gel, which was subsequently studied for skin tissue regeneration. In vivo experiments showed quick and good healing with sweat gland-like structures restored.

Similarly, Leong et al. [88] also used gelatin type A to fabricate hydrogel microspheres using a water-in-oil emulsion; however, the focus was to directly encapsulate and deliver non-ADCs instead of ADCs. Using chondrocytes as the model non-ADC type, a chondrocyte–gelatin type A suspension was stirred in soya oil and cooled down in an iced water bath. Chondrocyte-encapsulated gelatin microspheres of mainly 75–100 μm diameters were retrieved after two washes in phosphate-buffered saline (PBS) and suspended in an alginate hydrogel. Upon incubation at 37 °C, these gelatin microspheres melted, suspending chondrocytes within the microcavities for growth; the microspheres were hence termed

temperature-cured dissolvable gelatin microsphere-based cell carrier (tDGMC) [88]. Dense islets of rounded and viable cells with little dead cells were observed by 21 days in culture, and merging of cell islets throughout was noticed by day 35. This technique can be used to culture other non-ADCs as well; the dense cell islets can be obtained by dissolving the alginate hydrogel to yield microsized injectable cell islets.

The double emulsion technique is more complicated as the innermost phase has to be thoroughly removed, but it allows the fabrication of hollow microspheres, which translates to less biomaterial, better diffusion, and more space for cell attachment and growth. Su et al. [89] proposed a novel microcarrier for ADCs that was derived from such a technique. Hollow gelatin spheres were firstly fabricated from the double oil-in-water-in-oil emulsion and then modified with a surface crosslinking process and removal of uncrosslinked material, resulting in a hollow open structure named "shell-structure cell microcarrier" (SSCM). Human fetal osteoblasts were used as the model ADC type—the cells were able to adhere onto and proliferate on both the external and internal walls of the microcarrier at a higher efficacy than control gelatin microspheres and express the osteogenic phenotype.

Another group aimed to develop neuronal cell-laden porous (sponge) microspheres for neural tissue engineering. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) microspheres were fabricated using double water-in-oil-in-water emulsion (W1/O/W2) and freeze-dried to remove the aqueous solvent [90]. Murine neuronal cell line PC12 cells, neural progenitor cells (NPCs), and primary cortical neurons were viable and supported on the microspheres, exhibiting extensive spreading morphology and neuronal proteins. NPC differentiation was promoted, while axon and dendrites of cortical neurons were observed to be discriminated as a sign of neuronal maturation and functionality. PHBV was proposed as a suitable biomaterial for neuronal cell-laden microspheres because of their biodegradability and slow degradation, which matches the relatively slow growth of neuronal cells. Poly(lactic-co-glycolic acid) (PLGA) porous microspheres were also fabricated using a similar emulsion method, except that the pores were created by gas foaming [91]. The W2 phase consisted of ammonium bicarbonate that formed ammonia and carbon dioxide gas bubbles upon evaporation, creating a highly porous structure in the microspheres.

To exploit the ECM-like architecture of fiber meshes, substantial progress on fibrous microcarriers has also been made. One of the most recent developments is the simple fabrication of hybrid nanofibrous network-containing hydrogel microparticles [92]. A nanofibrous PCL network is firstly fabricated by electrospinning and soaked in the photoinitiated crosslinkable PEGDA solution containing fibroblasts; subsequently, through photomasking for PEGDA polymerization and removal of excess solution as well as dissolution of non-encapsulated PCL nanofibers using chloroform, controllable shapes of polymerized PEGDA gels containing nanofibers and cells can be produced. The hybrid microcarrier was characterized for both protein and cell delivery, which were deemed favorable. Specifically, cell adhesion within the hydrogel was achieved without the need for chemical modification to include cell adhesion moieties on PEGDA

gels—fibroblasts exhibited a spreading and elongated morphology along the nanofibers of the hybrid microcarrier, whereas a rounded morphology was noted in the simple PEGDA gel.

4.3.2 Post-fabrication Modification for Cell Adhesion

For ADCs, proper cell adhesion is critical in determining the success of their delivery vehicles; otherwise, they may undergo *anoikis* and decrease the efficacy of cell delivery. Therefore, many studies have explored the post-fabrication incorporation of cell-adhesive moieties in cell delivery vehicles.

One of the major shortcomings of synthetic biomaterials is their biologically inertness, i.e., they do not support cell adhesion, which affects their survival and phenotype. They can be functionalized through coatings or grafting of ECM proteins and ligands known to promote cell adhesion, e.g., fibronectin and laminin [59]. One of the most popularly incorporated bioactive ligands is the RGD sequence derived from fibronectin. It is known that RGD is the minimal recognition and binding sequence of cells' integrins to fibronectin and can support adhesion and spreading of cells [93, 94]. Differentiated SMCs were demonstrated to express the contractile phenotype in PEGDA hydrogels coated with fibronectin or laminin, or functionalized with linear RGD sequences [95]. Cyclic RGD have been shown to have higher affinity and selectivity toward cell binding, as well as greater stability against enzymatic degradation [96]. Patel et al. [97] functionalized the synthetic polynorbornene hydrogel by grafting linear and cyclic RGD motifs and comparing between the two forms using HUVECs. The group found that cyclic RGD was significantly more efficient in promoting cell adhesion—not only was the minimal concentration of the cyclic form (0.05 %) required for cell adhesion 50 times lower than that required for linear RGD, the cyclic RGD-conjugated gel supported cell spreading within 15 min, while the other required at least 90 min to achieve a similar state.

Larger ECM molecules such as gelatin and elastin can also be coated onto the biomaterials following the formation of the vehicle structure. Human fibroblasts and osteoblasts (both of which are ADCs) were viable and functional throughout the culture period on novel gelatin-coated gellan gum microspheres [98]. A water-in-oil emulsion involving preheated FDA-approved gellan in peanut oil yielded microspheres upon cooling, onto which gelatin was then grafted. PCL sponges were permeated with elastin using carbon dioxide gas foaming and crosslinked with glutaraldehyde to create a mechanically suitable and cell-supportive scaffold for cartilage tissue engineering [99]. In vitro studies concluded that the integration of elastin not only increased the water content in the sponges, it was also able to support chondrocytes better than pure PCL sponges. As mentioned previously, laminin was coated onto electrospun fibers to present cell-adhesive moieties to implanted neural cells, mimicking the native laminin-rich environment [56]. The basic bioactive sequence isoleucine–lysine–valine–alanine–valine (IKVAV) that

interacts with neural cells and increases their adhesion and proliferation can also be isolated from laminin and conjugated onto biomaterials, such as self-assembled peptides [100, 101].

4.3.3 Controlling Cell Functionality Through Micropatterns

The materials' surface topography is able to directly interact with cells and influence cell adhesion and growth in a specific manner. As this is especially crucial for cells that require alignment to function, e.g., neurons, endothelial cells and SMCs, the effect of submicron levels of surface modification on cell behavior has gained attention and been incorporated in various biomaterials [102]. The patterns are popularly achieved through photomasking, casting, and lithography techniques, which allow the width and depth to be tuned to control cell behavior and growth.

Endothelial cells in the native vascular environment are stretched and aligned along the longitudinal axis of blood vessels, i.e., parallel to blood flow. When organized in this orientation on manufactured biomimetic scaffolds, endothelial cells were able to retain their morphology and cell-specific gene expressions and possess athero-resistant qualities with less adhesion of platelets and monocytes [103]. Nikkhah et al. [104] recently demonstrated the importance of topography in directing endothelial cell adhesion and attachment with the maintenance of its functionality in vascularization. Longitudinal tubes of photoinitiated crosslinkable gelatin methacrylate hydrogel were fabricated with micropatterns by exposing selective areas to light using a mask (photomasking) [104]. HUVECs were of an elongated and spreading morphology and reorganized themselves to align along the length of the tubes by day 5, forming a 3D stable cord-like structure in 15 days. On the other hand, HUVECs on the unpatterned gelatin methacrylate gel block were randomly aligned, therefore affirming the effect of topography on cell behavior and functionality. Another study by Liu et al. [105] micropatterned the collector via lithography, hence fabricating electrospun fibrous meshes with specific grooves and ridges. Fibroblasts seeded on the micropatterned fibrous meshes were observed in higher numbers in the ridges and secreted ECM with similar patterns. Uttayarat et al. [106] used spin casting to fabricate microsized grooves in the lumen of polyurethane (PU) fibrous tubes to guide endothelial cell orientation in a vascular tube. The cells were observed to reach a confluent monolayer with alignment along the micropatterned topography, while retaining cell phenotype.

To guide neuronal cell growth and their neurite outgrowth as neural replacements (for example, at the interface of neurons and prosthetic equipment for successful and conserved transmission of stimulus), micropatterning serves as a favorable and simple microsized guidance. As demonstrated by Tuft et al. [107], photomask-fabricated indents (slopes instead of defined edges of grooves) on a methacrylated substrate on which neurite and Schwann cells were subsequently seeded and shown to be aligned. Not only can surface topography guide cell

alignment, proteins (i.e., biochemical cues) can also be specifically patterned to influence cell adhesion and their orientation. For example, neuronal cells were strictly confined on 5–10-μm-thick lines of cell-adhesive collagen patterned through microcontact printing on tissue culture polystyrene (TCPS) surfaces, which were backfilled with cell-repelling poly-L-lysine-g-polyethylene glycol [108]. The cells and their nuclei were also aligned parallel to the lines. On 5-μm-thick collagen patterns, neurite extension was significantly enhanced compared to non-patterned surfaces; neurite outgrowth was further stimulated by adding soluble retinoic acid in the cell culture medium.

4.3.4 Biomaterial-Free Cell Delivery

Ultimately, biomaterials have to be removed prior to implantation in order to completely eliminate the possible risks of biomaterial-related problems—mismatch of degradation rate to that of tissue development, immune responses, and toxic degradation products. Yet, direct injection of therapeutic cells may not be an efficient and effective cell delivery method since cells are not retained within the target site (leakage) and survive poorly without proper homing [109, 110]. This can be achieved by transplanting biomaterial-free microtissues, which have been cultured in vitro using biomaterials as a temporary scaffolding system. In other words, cells can be firstly cultured on biomaterials in the laboratory for a predetermined duration until they form microsized pieces of tissue comprising cells held together by their secreted ECM; subsequently, they can be removed from the biomaterial-based scaffolding system for implantation in the target site.

A confluent monolayer of cells, known as cell sheets, can be easily engineered in tissue culture plates by making use of responsive biomaterial surfaces to force the detachment of cells from the biomaterial surface without disrupting the cell–cell interactions and ECM holding the cells together. This strategy was pioneered by Kushida et al. [111]: Temperature-responsive poly(N-isopropylacrylamide) (PIPAAm) was coated on TCPS surfaces, and cells were grown on this coating. At the physiological temperature of cell culture, i.e., 37 °C, the coating is mildly hydrophobic, which is suitable for cell adhesion and culture; upon lowering the temperature to below 32 °C (its lower critical solution temperature, LCST), the coating becomes hydrophilic and the cells are upheaved from the surface. This produces a biomaterial-free cell sheet held together by the cells' secreted ECM, which is removable from the attached surface without any enzymatic treatment that would have also broken down the ECM [112, 113]. The PIPAAm coating is currently commercially available as UpCell® and has been utilized in fabricating cell sheets of various cell types, including mesenchymal stem cells [114] and fibroblasts [115]. A monolayer of mesenchymal stem cells implanted into infarcted myocardia of rats was able to reverse the thinning of the scarred myocardial wall while promoting vascularization and differentiating into vascular cells, with the overall effect of improving function of the infarcted heart [114]. Other cell sheet

fabrication strategies include the use of magnetic force and polyelectrolytes [116]. Ito et al. [117] developed a magnetic nanoparticle—RGD-conjugated magnetite cationic liposomes (MCL)—that coated surfaces by applying magnetic force beneath the surface; fibroblasts as model cells were able to adhere to the RGD-conjugated surface coating, and upon removal of the magnetic force, the cell monolayer was detached. Kito et al. [118] successfully utilized a similar MCL strategy to retrieve an iPSC monolayer for implantation to induce angiogenesis in ischemic tissues, except that MCLs were labeled in the cells instead of on a surface coating. Likewise, cells can be detached from RGD-conjugated polyelectrolyte coatings via altering the polarization [119, 120].

The difficulty in handling an extremely fragile monolayer of cells is a severe limitation of cell sheets in the clinical setting. Furthermore, being monolayer, it is difficult to scale up to form and replace a 3D macrosized tissue defect since stacking multiple layers of monolayers is a laborious process. Biomaterial-free 3D systems are favorable for cell delivery as they can deliver a large amount of cells without unwanted leakage to non-target sites and circumventing any biomaterial-related problems. As an example, the engineered PTCC system for hyaline cartilage tissue engineering (mentioned in Sect. 4.2.1, Gong et al.) was further improvised by Su et al. [121] by replacing temperature-responsive agarose with ionic-responsive alginate as the gel bulk. Alginate, which crosslinks in the presence of calcium divalent ions, can be completely and quickly removed by immersing the scaffold in a sodium citrate-containing solution, whereby citrate ions act as calcium-chelating agents. After 35 days of culture, the extensive and dense ECM network secreted by chondrocytes was able to support the construct such that alginate was removed without a collapse in structure (compared to a conventional cell-laden hydrogel without microcavities), thereby leaving behind a hyaline cartilaginous construct free of biomaterials. Implantation into rabbit osteochondral defects in the knee joint showed good integration with host tissue and expression of hyaline cartilage phenotype with no visible fibrosis.

4.4 Conclusion

Cells interact with the surroundings; the biochemical and mechanical cues they receive from the surroundings are translated and lead to modification of cell behavior. Without proper cell adhesion in certain cells (ADCs), not only will the cell phenotype be affected, a specific apoptotic process named *anoikis* may also occur. Therefore, the importance of biomaterial properties and design cannot be further emphasized. In this chapter, the four basic cell delivery structures hydrogels, fibrous meshes, sponges, and decellularized ECM and their recent developments in cell delivery have been discussed. While hydrogels are more naturally suited for soft tissues and for delivery of non-ADCs, they have been modified to possess cell adhesion moieties that allow ADCs to be successfully delivered using hydrogels as well. Fibrous meshes, with their similarity to ECM architecture, have

been widely used for the delivery of many cell types. Developing aligned fibers for guiding cell growth and behavior has been the recent focus of electrospun meshes. Sponges are highly porous scaffolds in which ADCs adhere to walls of the pores; therefore, porosity and the interconnectivity of pores are important design factors. Finally, decellularized scaffolds, derived from biological tissues, offer site-specific ECM proteins to guide the newly seeded cells. They have been solubilized and converted into sponges and hydrogels of controllable shapes and sizes.

Cell delivery vehicles have been greatly improvised to better mimic the microenvironment of cells thus far. In order to create an ideal cell delivery vehicle, both biomaterials and structure have been researched on. Biomaterials are blended and copolymerized in order to offset the shortcomings and combine the advantages of the individual biomaterials. Also, modification of surfaces with cell adhesion moieties and ECM molecules and surface topography has become an established technique to maintain functionality of delivered cells. While some hydrogels are injectable, other structures (sponges and fibrous meshes) have also become injectable by downscaling them into microsized particles. Furthermore, biomaterials have been exploited as temporary scaffolding systems for in vitro culture to create micro- or macrosized biomaterial-free tissues for implantation, hence eliminating risks of biomaterial-related complications in vivo. This variety of improvisations to conventional cell delivery vehicles has brought us one step closer from the bench to the bedside.

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