Cell encapsulation via microtechnologies

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Review

Cell encapsulation via microtechnologies

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ABSTRACT

The encapsulation of living cells in a variety of soft polymers or hydrogels is important, particularly, for the rehabilitation of functional tissues capable of repairing or replacing damaged organs. Cellular encapsulation segregates cells from the surrounding tissue to protect the implanted cell from the recipient's immune system after transplantation. Diverse hydrogel membranes have been popularly used as encapsulating materials and permit the diffusion of gas, nutrients, wastes and therapeutic products smoothly. This review describes a variety of methods that have been developed to achieve cellular encapsulation using microscale platform. Microtechnologies have been adopted to precisely control the encapsulated cell number, size and shape of a cell-laden polymer structure. We provide a brief overview of recent microtechnology-based cell encapsulation methods, with a detailed description of the relevant processes. Finally, we discuss the current challenges and future directions likely to be taken by cell microencapsulation approaches toward tissue engineering and cell therapy applications.

1. Introduction

Technologies for creating living functional tissues in the laboratory for the repair or replacement of damaged organs have emerged as new promising tools for therapeutics. Implanted cells must be protected from attack by the host immune system. Currently, the most common immune protection techniques have involved entrapping therapeutic cells in a polymeric coating using diverse hydrogels [1–4]. Several technological barriers must be addressed before these approaches may be applied practically and widely. The selection of a suitable encapsulating material with appropriate porosity, which can facilitate the transport of nutrients, proteins, DNA, and drugs while blocking attack of antibodies and immune cells, is critical. The capsule must be mechanically stable and easy to handle. These requirements may be satisfied by controlling the pore size and thickness of the encapsulating polymer membrane at the microscale ranges. The cell viability and metabolic status have been shown to be optimal if the encapsulated cells are on the order of one hundred microns in size. [5] Although several encapsulation methods have been developed, the satisfaction of all of these requirements still remains challenging [6].

Advances in recent microfabrication techniques based on photolithography have been widely adopted in the biomedical fields and have enabled the preparation of devices or systems that remove some of the bottlenecks that have historically slowed certain processes in the life sciences. Some of these technologies have faced barriers to adoption in the biomedical fields due to the high costs, long time required for microfabrication processes, limitations on the materials, and requirement of complicated facility and labor skills. Softlithography has circumvented many of the problems associated with conventional silicon-based photolithography processes and has enabled the emerging of strong cell encapsulation tool [7–9]. The microsystems based on softlithography present several major advantages: small quantities of reagents and sample volumes are needed, the experimental timescales are short, the processes are cost-effective, a diversity of materials may be used, and the dimension of experimental platform is reduced enough to be placed in the cell-incubator. The ability to handle small sample volumes using a microscale platform permits precise control over the number and sizes of encapsulated cells, as well as over the shape of the cell-laden polymer structure.

In this review, we describe a variety of methods that have been developed for cell encapsulation based on microscale platforms. Microsystems can encapsulate cells in diverse shapes, including beads, sheets, and fibers, and can finely control the sizes and numbers of encapsulated cells on the microscale. The materials needed for microtechnology-based cell encapsulation and a detailed description of the microencapsulation processes are described. Finally, we discuss the current challenges and future

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opportunities made available by cell microencapsulation for tissue engineering and cell therapy applications.

2. Concept of cell encapsulation and overview of encapsulation techniques based on macro-platforms

Transplanted cells are recognized by host immune cells as foreign substances. Like antigens, transplanted cells tend to trigger an immune response. Activated immune cells, such as macrophages, granulocytes, lymphocytes, and fibroblasts, secrete cytotoxic molecules and cytokines that cause the structural and functional loosing of implanted cells [10,11]. Cells for which a donor shortage exists and that are unable to grow in artificial media, for example, pancreatic islet cells, must be provided from xeno or allo species. A host immune response and the associated functional damage to implanted cells may be avoided by encapsulating the cells in a variety of non-cytotoxic and semipermeable hydrogel. The most important requirements of cell encapsulation is that the cells retain their function and release cytokines or hormones in the capsule, and they are also protected from a patient's immune system (Fig. 1) [12,13]. Therefore, the cell encapsulation method is crucial and encapsulating hydrogel membranes should allow oxygen and nutrients to reach the internal cells, while excreted wastes and therapeutic products may be released. The encapsulation methods could be classified into macro- and micro-platform based method. Macro platform based method involves encapsulation of cells in hollow fiber and bulk hydrogel using macroscale devices. In contrast, micro-platform based method involves the encapsulation of cells in microparticles and microfibers using microfluidic chips and micromolding (Fig. 1). Encapsulation of cells in bulk hydrogels offer the simplest encapsulation method, with a process involving the steps of: 1) suspending cells at a desired density in a pre-gel solution; 2) injecting the suspension into the container; and 3) gelling the pre-gel cellular suspension via a temperature transition, chemical reaction, or photocuring process [14]. The ultrasonication-induced gelation method reported by Wang et al. [15] is an additional process that accelerate gelation kinetics. Synthetic polymers, such as poly (ethylene glycol) (PEG)-based hydrogels, are commonly used as bulky hydrogel encapsulating materials [16–27]. Most of PEG-based hydrogels for encapsulation require a photopolymerization procedure for gelation. Mixtures of monomers, crosslinkers, and photoinitiators are essential components of gel formation [28]. PEG hydrogels that form via a temperature transition have been shown by some studies to display advantages over photopolymerization reactions when applied to areas that permit only limited light penetration [16,17]. Foo et al. reported a two-component molecular recognition gelation method in which cells are encapsulated without environmental condition changes (e.g., temperature, pH, or ironic strength). They developed a crosslinking method that linked multiple repeats of conserved tryptophan residues and proline-rich peptide domains in a sol to form a gel phase upon mixing [29]. The WW domains (named

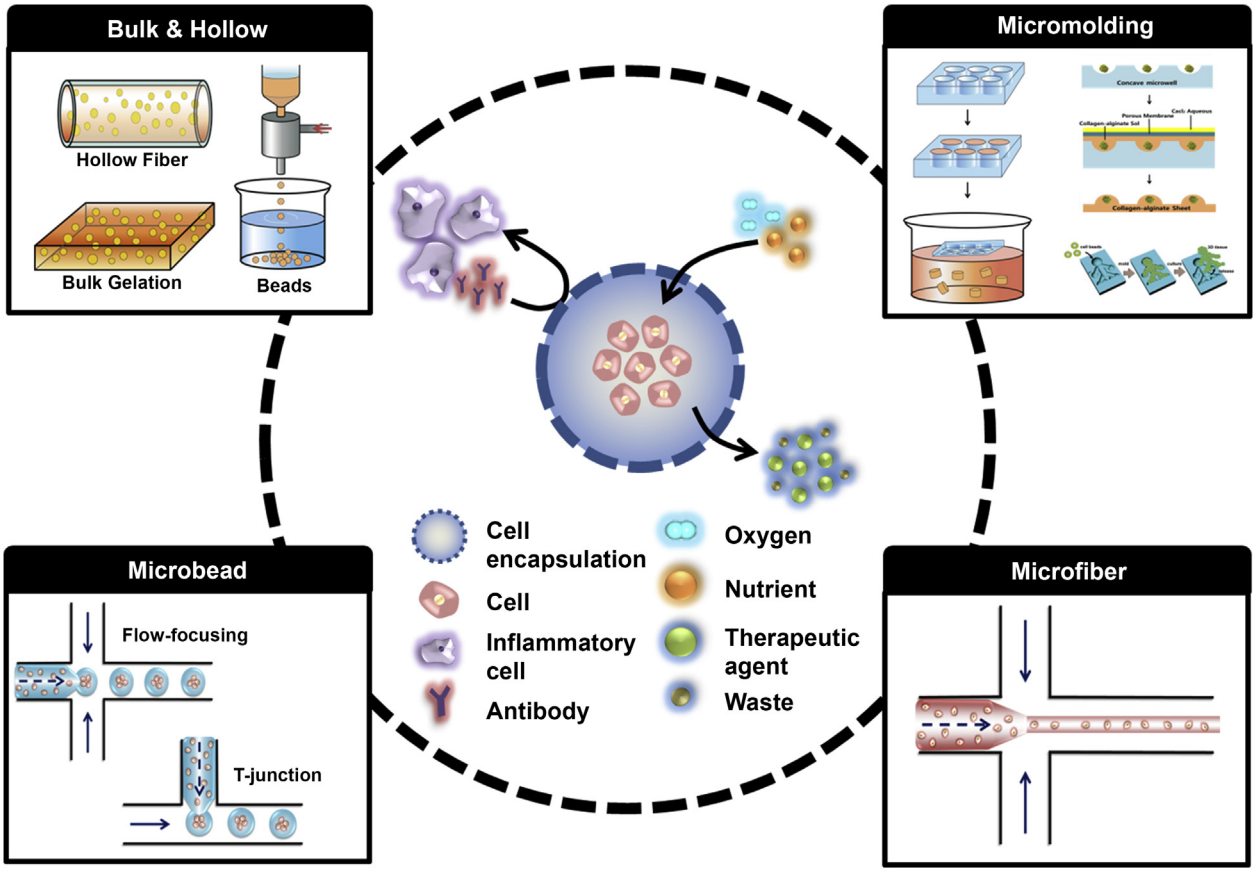

Fig. 1. Schematic diagram and a list of the major methods used to achieve cell encapsulation: Cellular encapsulation sequesters the encapsulated cells from inflammatory cells and antibodies without impeding the inward diffusion of oxygen and nutrients and the outward diffusion of therapeutic agents and wastes. (Inspired by Orive et al. [12]) The major methods used for cell encapsulation are the macro platform (mainly nozzle-based platforms), micromolding (The figure on the left was inspired by McGuigan et al. [84] and the upper figure on the right was inspired by Lee et al. [89]. The bottom figure was reprinted from Matsunaga et al. [86]: Molding cell beads for rapid construction of macroscopic 3D tissue architecture. Advanced Healthcare Materials. 2011. 23. H90–H94. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission), microfluidics-based microbeads, and microfibers.

Table 1
Methods, cell type, materials, and their tissue engineering applications involving cell encapsulation via macroencapsulation techniques.

Methods	Materials	Cell type	Ref.
Bulk Hydrogel	PEG-based hydrogel	MSCs	[16,17, 19–21]
		Chondrocytes	[23–26]
		Fibroblasts	[22]
		ESCs-derived motor neurons	[27]
	Photopolymerizable styrenated gelatin	Chondrocytes	[112]
	Silk fibroin	MSCs	[15]
	Photopolymerized ELP	ADSCs	[113]
	Chitosan	Chondrocytes	[114]
	Photopolymerized HA, collagen, and laminin	Schwann cells	[115]
	D-mannitol crystals with photocrosslinkable MAC	Neural stem/progenitor cells	[116]
	MITCHs	PC12, HUVEC, and murine adult neural stem cells.	[29]
	ADA, gelatin, borax	Hepatocytes	[117]
Hollow fiber	PAN-PVC	PC12	[35]
		BHK-GDNF, PC12, PC12A	[36]
		BHK-hNGF	[37]
		PC12, Bovine adrenal chromaffin cells	[38]
		Pancreatic islets	[42–45]
	Chitosan, Alginate in PAN-PVC	R208N.8, R208F, PC12	[33]
	Polysulfone	BHK-GDNF	[34]
	Collagen in polysulfone fiber	Hepatocytes	[39–41]
Beads	Alginate, gelatin	Embryonic stem cells	[118]
	Collagen, HEMA-MMA-MAA terpolymer	Hepatocytes	[119–121]
	Alginate, polylysine	Pancreatic islets	[50,54,66, 122,123]
		Fibroblasts	[124]
		Bone marrow cells	[125]
		PC12	[56]
		Sperm	[64]
		BHK, transfected to produce hVEGF	[60]
	Alginate, Chitosan	Fibroblasts (R208N.8, R208F), PC12	[33]
	Agarose	Pancreatic β-cell line (MIN6)	[126]

because of single-letter amino acid symbol of tryptophan: W) and proline peptides were bonded to form a hetero-assembly structure in which multiple repeats were linked by hydrophilic spacers. Synthetic hollow thermoplastic fibers that form tubular-shaped diffusion chambers have been used for cell encapsulation. The hydrogel matrix or culture medium was shown to immobilize cells in the fiber and physically isolate them from the surrounding tissue [6,30]. Hollow fibers are fabricated by extruding a polymer solution that is pumped through an outer region of the nozzle and an aqueous solution flew through a central lumen. Fabricated hollow fibers are collected in an aqueous water bath, and the solvent is washed away. Macroencapsulation is then accomplished by sealing the hollow fiber [31]. Membranes with 0.5–4.8 mm of thickness are typically used for this procedure [32]. Encapsulation techniques using a hollow fiber method are widely used, especially for encapsulating neuronal growth factors secreting or dopamine-secreting cells [33–38], hepatocytes [39–41], or pancreatic islets [42–45]. Macroencapsulation systems have advantages in that the gelation procedures are relatively easy and the gels can be retrieved easily. One of the challenges involved in using macroencapsulation approaches is that the membrane tends to be broken under mechanical forces. Thin membranes are desirable because they present short diffusion barriers; however, the membranes may be easily broken, leading to a fibrotic reaction or blood clotting. Additional challenges include limitations on the diffusion-controlled oxygen and nutrient supply and the accumulation of waste products in the capsules. Large capsules were found to contain a central necrotic core after implantation due to nutritional deficiencies caused by the long diffusion path. The use of small hollow fibers may improve the nutrition conditions but may require longer or more numerous devices that increase patient discomfort post-implantation [32,46].

The sizes and shapes of an encapsulating material are important issues and can affect to the survival of implanted cells and the immune response of a recipient. Controlling the diffusion rate is central for maintaining cell viability [2], and spherical beads can offer good diffusion properties. The membrane forms the diffusion path between the cell and the outer environment, and this path is minimized in spherical beads that have a high surface-to-volume ratio. One study observed that smaller capsules provide much lower pericapsular cellular reaction in which cell adhesion to the surface of the encapsulating material was reduced compared to larger capsules [47]. The beads are mechanically stable in 3D and do not readily split. The encapsulated cells tend to retain their morphologies and functions, and the beads are especially advantageous for cells that require cluster formations, such as pancreatic islet cells [48,49]. These advantages have been demonstrated in a variety of bead encapsulation methods. Many types of cells, including pancreatic islet cells [48,50–55], neuron or neuron-like cells [33,56–59], and transfected fibroblast cells that produce human VEGF [60], have been encapsulated to achieve certain therapeutic purposes. The encapsulation methods described in this section were generated using macroscale system. Here, these methods will be referred to as macroencapsulation methods to contrast the formation of beads using microfluidic techniques. In the early 1980s, Lim and Sun proposed a novel islet macroencapsulation procedure in which cross-linked alginate was used to protect transplanted cells from the recipient's immune system [54]. Liquid–core encapsulation methods remain popular [61]. Beads containing islets may be fabricated using a syringe pump extrusion technique adapted from Ennis and James [62], and Sparks et al. reported modified method [63]. A droplet containing islet cells is dropped into a calcium chloride solution that triggers gel formation. Islet cells in the alginate microcapsule retained their morphology and function over 4 months under *in vitro* culture conditions. Intraperitoneally implanted encapsulated islets displayed prolonged survival and were found to preserve their normoglycemia state relative to the non-encapsulated islet control group upon injection into streptozotocin-injected diabetic rats. The same technique was used to perform several studies with different cell types. Bovine spermatozoa were encapsulated for use in artificial insemination by Nebel et al. [64] Winn et al. encapsulated dopamine-secreting cells (PC12) in sodium alginate [56]. In a study by Zielinski et al. [33], PC12 and two other fibroblast cell lines were encapsulated in cross-linked chitosan or sodium alginate beads using an air jet droplet generator to study the influence of the matrices on the growth of both types of anchor-dependent and -independent cell. Despite of extensive studies, the methods described above do not readily permit the precise control over capsule size, shape, strength, and permeability [65]. Wolters et al. developed an air jet droplet generator that produces small, uniform, and smooth alginate beads [66]. The generation system comprised an alginate reservoir and a droplet generating part connected by polyethylene tubing. This method used a microscale nozzle, a component of the sizer, and the drag force of a gas stream. Droplets were formed at the end of the nozzle as the alginate solution passed through the nozzle, and the

droplet detached from the nozzle upon application of the drag force. The bead size was determined by the size of the jacket, the gas flow rate, and the nozzle diameter. This method permitted the fabrication of beads having the intended length and diameter. This micro droplet generator was used by other groups to conduct a number of cell encapsulation studies [67]. De Vos et al. found that guluronic acid, a constituent of alginate, affected the biocompatibility of the encapsulated islet cells both *in vitro* and *in vivo* [50,51]. However, microcapsules generated using an air jet can suffer from several disadvantages. For example, the air jets can form "tails" on the microcapsules that can cause an immune response. The air flow applied during microcapsule formation can impose harsh shearing forces on the cells, and small air bubbles trapped in the alginate can limit the diffusion of substances and reduce the stability. The air flow in a device can be adjusted, and bubbles and tails can be minimized, by the application of sinusoidal vibrations and electrostatic potentials. These approaches are only applicable to low-viscosity alginates [68,69] (Table 1).

3. Microfluidics-based encapsulation of cells in microbeads

The problems associated with macroencapsulation methods could be overcome by employing microtechnologies that have several advantages over the macroscale platforms. Microtechnologies permit a high degree of control over the morphological and dimensional properties, cost-effective process, and the experimental platforms that are physically smaller than the macroencapsulation systems. Cells are typically encapsulated by gelling cell suspension in a hydrogel solution to form a solidified matrix. The gelation mechanism can vary with the encapsulating material. Thermally sensitive gels (e.g., gelatin or agarose) are formed through a temperature change. Photoresistive (e.g., poly(ethylene glycol (PEG))) and ion-based (e.g., alginate) crosslinking materials are solidified by light (e.g., UV) and divalent ion (e.g., Ca^{2+}), respectively. Gelation via a temperature change or UV exposure can influence the cell viability or physiology. Thus, gelation methods using ion-based crosslinking tend to be safer for cell encapsulation. The biocompatibility and biodegradability of an encapsulating material is very important. Natural polymers, such as alginate, agarose, collagen, and chitosan, and synthetic polymers, such as PEG, poly(lactic-glycolic acid) (PLGA), poly(lactic acid) (PLA), and poly(glycolic acid (PGA), are well known to be suitable encapsulation materials. Amongst them, alginate which is generally used for biomedical applications, such as cartilage or bone regeneration and diabetes treatments, is most frequently used because of its simple and rapid gelation process, its excellent biocompatibility and biodegradability. Microbead fabrication methods based on microscale platform may be classified into two major approaches: flow-focusing and T-junction bead formation. Flow-focusing, as shown in Fig. 1 of the microbead section, forms microbeads by allowing a core fluid to be surrounded by sheath stream flowing. In contrast, T-junctions form microbeads by permitting the core fluid to be swept away by one sheath stream in one direction. A summary of the microfluidic technologies used for microbead cell encapsulation is listed in Table 2. Haeberle et al. [70] presented a method for the centrifugally induced fabrication of microscale and calcium ion-hardened alginate beads using polymer micro-nozzles. Their study added cell-laden microbead fabrication capabilities to a centrifugal multiphase microfluidic platform [71]. The micro-nozzle was mounted on a centrifuge rotor and cell-laden alginate droplets were dispensed horizontally by centrifugally induced artificial gravity. The droplets were gathered into a $CaCl_2$ solution-containing tube that hung on the end of the rotor. The collected alginate droplets underwent a hardening process to form calcium alginate beads. The bead diameter could be adjusted between 180 and 800 μm according to the nozzle geometry and the rotational speed. Their centrifugal bead encapsulation method is pulse-free and, thus, reproducibly produces droplets under the induced artificial gravity conditions (Fig. 2(A)). Tsuda et al. [72] presented monodisperse cell-laden microbeads made of a self-assembling peptide (SAP; PuraMatrix RADA 16) gel for 3D cell culture by using stereolithography (Fig. 2(B)). They encapsulated bovine carotid artery endothelial cells using a previously reported 3D microfluidic axisymmetric flow-focusing device using an external gelation method [73,74]. Pre-gel SAP solution droplets were gelated after contacted the crosslinking agent in a continuous phase. With this method, they could control over the size of the beads in microscale easily and obtain uniformly reproducible cellular microenvironment by tuning the flow rate ratio. Tan et al. [75] described the fabrication of monodisperse alginate microbeads using a droplet-forming T-junction microfluidic channel. Their device permitted control over the bead size through adjustment of flow rate. The calcium carbonate nanoparticles in the alginate solution induced internal gelation of the microbeads. The alginate droplets that

Table 2
Summary of microfluidic technologies used for cell encapsulation in microbeads.

Methods			Materials	Cell type	Ref.
Emulsification	PDMS-based microfluidic chip	Flow-focusing	Polystyrene	HL60	[127]
			Alginate	P19 EC, MCF7, HepG2	[128]
				P19 EC	[129]
				HeLa cells	[130]
			Alginate, agarose	Eukaryotic cells, Sertoli cells	[131]
			PEG	C2C12 (p7), Placenta-derived human MSC/ESC	[132]
			PFPE-PEG	2C6 hybridoma cells	[133]
			Agarose	*Escherichia coli* cell	[134]
		T-junction	Agarose	mES cells	[135]
			Alginate	HepG2	[76]
				Yeast cell	[136]
				Mammalian cell	[75]
		'on the fly'	4-HBA	Yeast cell	[137]
		Drop	Alginate	Chondrocyte	[138]
	Axisymmetric Flow-Focusing Devices	Flow-focusing	SAP (self-assembling peptides)	Bovine carotid artery endothelial cells	[72]
			Alginate	Chlamydomonas	[74]
Microfabricated nozzle	Micro-nozzle	Micro- airflow-nozzle	APA (alginate-poly-L-lysine-alginate)	CHO	[139]
		Micro-nozzle array	Alginate	Human kidney 293 cells	[140]

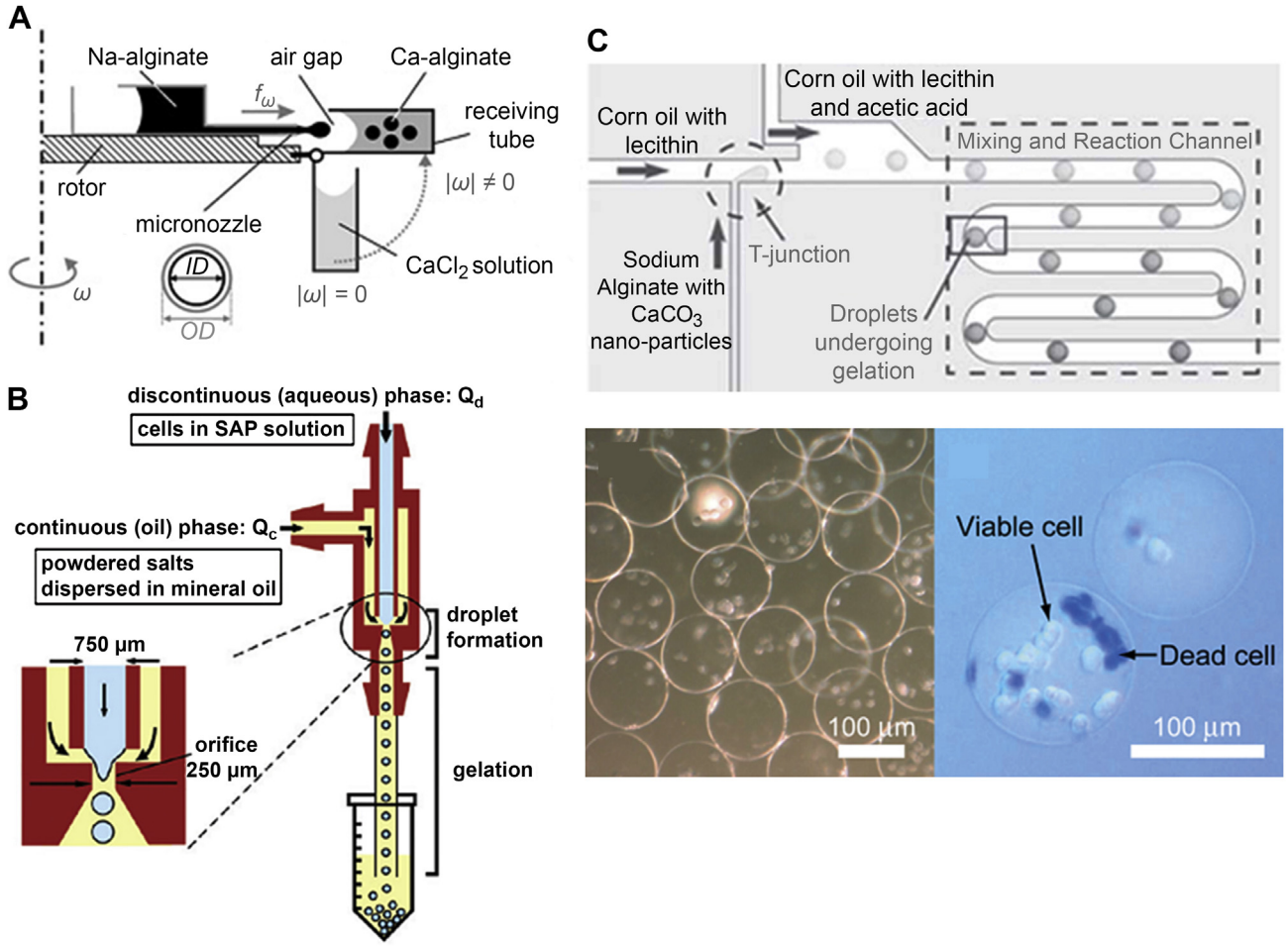

Fig. 2. Representative microbead fabrication methods for cell encapsulation. (A) A cell encapsulation method for the centrifugally induced fabrication of alginate microbeads using polymer micro-nozzles. The micro-nozzle is mounted on a centrifuge rotor and cell-laden alginate droplets are dispensed horizontally by centrifugally induced artificial gravity. (Haeberle et al., Journal of Microencapsulation, 2008; 25 (4): 267–274, copyright© 2008, Informa Healthcare. Reproduced with permission of Informa Healthcare). (B) A monodisperse cell-laden microbead fabrication method by using axisymmetric flow-focusing devices. Pre-gel SAP solution droplets were gelated after contacted the crosslinking agent in a continuous phase. (Reprinted with permission from Tsuda Y, Morimoto Y, Takeuchi S. Monodisperse cell-encapsulating peptide microgel beads for 3D cell culture. Langmuir. 2009; 26:2645–9. Copyright 2009 American Chemical Society). (C) The fabrication of monodisperse alginate microbeads using droplet-forming T-junction microfluidic channel. The calcium carbonate nanoparticles in the alginate solution induces internal gelation of the microbeads and the alginate droplets that are formed at the T-junctions undergo gelation along the mixing and reaction channels. (Tan et al.: Monodisperse alginate hydrogel microbeads for cell encapsulation. Advanced Materials. 2007. 19. 2696–2701. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

formed at the T-junctions underwent gelation along the mixing and reaction channels (Fig. 2 (C)). Um et al. [76] described a method for generation of cell-contained hydrogel beads using a double T-junction microfluidic channel. The device was used to encapsulate HepG2 (hepatocellular carcinoma) cells in a PuraMatrix gel beads. The microchannel included 3 inlets through which the sol state pre-gel, the oil phase and the HepG2-containing medium were introduced respectively. The sol state PuraMatrix droplets were cross-linked by the ions in the cell medium. Care is needed when implementing this method because as the pH of the sol material is low (pH 3), and longer cell pretreatment times in the sol state may reduce the cell viability.

4. Encapsulation using micromolding methods

Cell-encapsulating particles could be generated by replication from a micromold. The micromolding method was used to fabricate complex cell-laden hydrogel shapes on a massive scale using specialized lithography equipment. Several studies reported the use of hydrogel micropatterning to achieve encapsulation. A patterned photomask was used to fabricate an array of regularly shaped microstructures [77–81]. Koh et al. [78] fabricated a fibroblast-encapsulating cylindrical PEG-based hydrogel structure. UV exposure through the photomask induced transient gelation in specific regions, and the unexposed areas were washed away leaving the intended constructs. A similar method was used by Du et al. [79] to fabricate more complicated microstructures. The hydrophobic effects drove the “lock-and-key” assembly of microgel units to form cross- or rod-shaped gel units that contained cells (Fig. 3(A)). Another common approach to the formation of microstructure involves the use of a micropatterned mold. Khademhosseini et al. [82] encapsulated cells in methacrylated hyaluronic acid (HA) micropatterned hydrogels. The HA precursor solution containing the cells and photoinitiator was molded by the patterned PDMS stamp. The trapped HA solution was photo-cured by UV light and the PDMS mold was removed. An array of cell-laden microstructure was successfully formed using a simple micromolding method (Fig. 3(B)). Shepard et al. created patterned channels designed to support the growth of neurites [83]. A PEG precursor solution containing dorsal root ganglia HT-1080 cells was introduced into the patterned PDMS channels and gelled. The channel walls of the hydrogel physically guided the extending

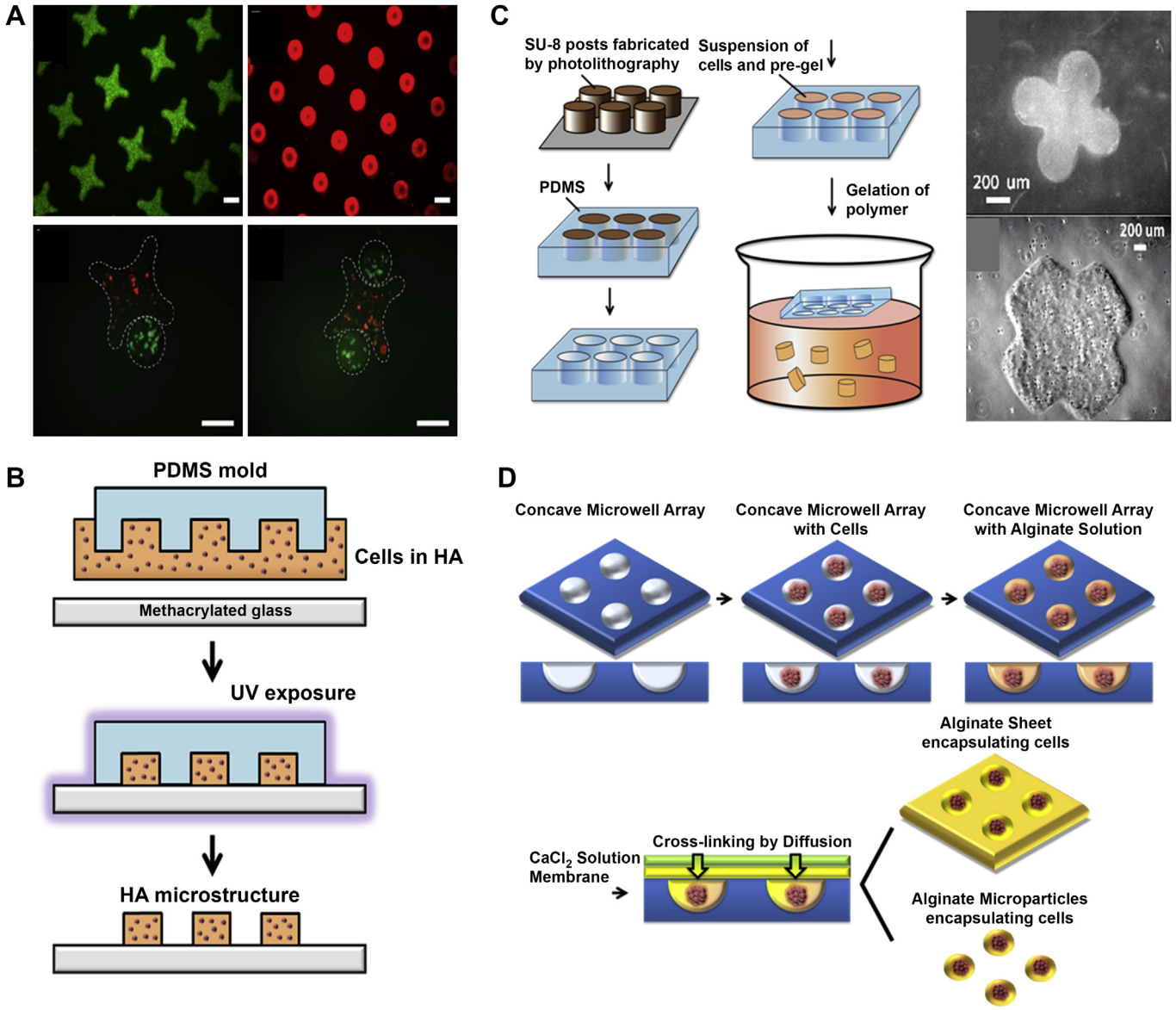

Fig. 3. Cell encapsulation using micromolding methods. (A) Direct assembly of "lock-and-key" designed microgels. By using photomask-patterning method, regular cross- and rod-shaped microgels are possibly constructed. (Reprinted from Du et al. [79] PNAS – Copyright 2008. The National Academy of Sciences of the United States of America). (B) By stamping micropatterned PDMS mold on HA solution, desired array of cell-laden microstructure was formed. (Inspired by Khademhosseini et al. [82]) (C) Schematic diagram showing the formation of cell-embedded micromodules formed by using a photolithographically fabricated PDMS template. (Inspired and reprinted from McGuigan et al. [84] Langmuir – Copyright© 2008 McGuigan et al.). (D) Schematic illustration of the encapsulating cell spheroid method on concave microwell array. The morphologies of cell sheet and hemispheric particles containing spheroids formed in concave micromolds. (Inspired by Lee et al. [88] and Lee et al. [89] Relevant products are available at www.microFIT.kr).

neurites. McGuigan et al. [84] and Bruzewicz et al. [85] have described encapsulation methods involving the use of PDMS membrane templates (Fig. 3(C)). A PDMS membrane is fabricated by pouring a pre-polymer onto a silicon wafer bearing lithographically prepared SU-8 posts. A hydrogel solution containing cells was then loaded into the holes of the membrane, and after gelation, the particles were released into the cell medium by gentle shaking. The formation of macroscale three-dimensional structure from the "bottom-up" fabrication method was proposed by Matsunaga et al. [86] Complex millimeter-thick tissues could be obtained by stacking a number of "cell beads" fabricated using axisymmetric flow-focusing devices in a PDMS mold chamber. Several hours after seeding the cell beads into the mold, the beads contacted and shrunk compactly into the designed shape. A rolled-up alginate sheet was shown to act as a bioartificial pancreas [87]. Pancreatic β-cells containing self-assembled microcontainers were attached to the alginate sheet, and the sheet was rolled up to form a cylinder. Nano-sized pores were placed on the walls of the microcontainer, to permit small molecules to diffuse. The functional capacity of the encapsulated β-cells was demonstrated by ELISA.

Micromolding methods enable the formation of cell spheroids and the *in situ* encapsulation of spheroids on the same platform. Aggregated cells tend to be more stable, viable, and functional than single cell culture due to the effects of cell–cell contact. Cell spheroids could potentially be widely applied in biomedical areas, and a variety of studies have attempted to produce cell spheroids of uniform size and shape. The employment of cell spheroids in cell-based therapy requires simple and safe encapsulation methods. Lee et al. [88] presented a process of spheroid formation and *in situ* encapsulation. A hemispherical PDMS micromold was used to form size-controlled spheroids, and a nanoporous membrane was used to control the diffusion of a crosslinking agent. Uniformly sized

Table 3
Summary of microfluidic technologies used for cell encapsulation of cells via micromolding techniques.

Methods	Materials	Cell type	Ref.
Micropatterning using photomask	PEG-DA	NIH 3T3	[78], [81]
	PEG-MA	NIH 3T3	[79]
	GelMA	NIH 3T3, HUVEC	[77]
		NIH 3T3, HUVEC, C2C12, cardiac stem cells	[80]
Micropatterning using patterned PDMS	Methacrylated HA PEG	NIH 3T3, ESR1	[82]
		Dorsal root ganglia explants, HT-1080	[83]
Replica molding	Collagen, Matrigel™, Agarose	NIH 3T3, HepG2, cardiomyocytes	[84]
		NIH 3T3, HepG2	[85]
Molding cell beads	Collagen	NIH 3T3, HUVEC or HepG2	[86]
Microcontainers on rolled-up sheet	Gold-electroplated nickel, alginate	Pancreatic β-cells	[87]
Microconcave well and nanoporous membrane	Alginate	HepG2	[88]
	Collagen-alginate composite	Pancreatic islets	[89]

HepG2 cells were obtained by seeding cells in the concave wells. The spheroids were encapsulated with alginate without replating, which could be advantageous by minimizing damage to cells. The particles were formed by spreading alginate onto a concave microwell array containing islet spheroids, and a porous membrane was placed on the pre-gel solution. The transport of the cross-linking agent, aqueous $CaCl_2$, through the porous membrane induced slow gelation of the alginate, and the encapsulated spheroids maintained high cell viability. Slight modifications to Lee's method [88], Lee et al. [89] suggested a method for the *in situ* encapsulation of islet-like spheroids in a sheet form (Fig. 3(D)). Concave wells 300 μm in diameter were used to form small-volume islet spheroids and eliminate the central necrosis problems. Sodium alginate and collagen-alginate composite (CAC) were used as the encapsulation materials. The suitability of these materials was evaluated *in vivo*. Encapsulated islet spheroids functioned well to ameliorate chemically induced diabetes in mice over a 4 week study, enabling the achievement of normal glucose levels below 200 mg/dL. A summary of the cell encapsulation via micromolding techniques is listed in Table 3 below.

5. Microfluidics encapsulation of cells in microfibers

Encapsulation of cells in microfibers has several advantages to bead- and sheet-based cell encapsulation methods. Microfibers are easy to handle, whereas beads are difficult to handle and sheets requires careful handling and has difficulty in implantation due to size. Diverse cells could be encapsulated into a microfiber, and a 3D scaffold can be fabricated simultaneously. The fibrous shapes may be readily used to build porous large 3D tissue structures that facilitate nutrient and oxygen diffusion [90]. Microfluidic spinning is defined as a formation of fibers in a microchannel using the co-axial flow of pre-polymer and crosslinking agent. This method is similar to the wet spinning [91] that the crosslinking agent is supplied directly by the coaxial flow instead of by the bath. Microfluidic-spun fibers have been formed using a variety polymers, including alginate [92–94], chitosan, poly(lactic-co-glycolic acid (PLGA) [95,96], and chitosan/alginate [97]. The central advantages of microfluidic spinning methods are that the diameter of the fiber can be readily tuned from a few to a few hundred microns by regulating flow rate only, and that a diversity of cells can be encapsulated without incurring significant damage to the cells. Microfluidic spinning is the most suitable fiber formation technique for cell encapsulation because this technique does not require high voltages or temperatures, fibers can be fabricated continuously, and the diameters of fiber can be controlled so as to be small but large enough to encapsulate cells. Table 4 summarizes the methods, materials, cell types, and the tissue engineering applications of cell encapsulation using microfiber techniques.

The most common biomaterial used for cell encapsulation by microfluidic spinning is alginate. Alginate has a number of advantages over other biological hydrogels, including hyaluronic acid, chitosan, agar, and fibrin, that it is economic, and easy to form fiber using microfluidic devices [98]. PDMS-based microfluidic chips and glass-based microfluidic chips have been used for microfluidic spinning. Both types of devices can generate a coaxial flow for spinning and easy extrusion of a fiber. Although the materials and methods are quite similar, the cell types used in cell encapsulation applications have been diverse. Suigiura et al. [99] used human kidney cells to encapsulate cells, and they noted that the cell encapsulation into microfibers permits high levels of nutrient and oxygen transport and a high cell activity, all of which benefit tissue engineering, cell transplantation, and drug delivery applications. Lee et al. [98] fabricated alginate hollow fibers and encapsulated HIVE-78 cells to mimic the blood vessels that are central to gas and nutrient delivery in tissues (Fig. 4(A)). Both Suigiura et al. and Lee et al. tried to form microvasculature structures using microfibers. Kang et al. [100] developed a system for generating spatially coded microfibers using a valve system to control the flows in the microchannels. They encapsulated hepatocytes and fibroblasts (L929) serially in a single fiber as well as horizontally across a fiber bundle. They expect that this technique will be very useful for

Table 4
Summary of microfluidic technologies used for cell encapsulation in microfibers.

Methods	Materials	Cell type	Ref.
Microfabricated Nozzle Array	Alginate	Human kidney 293 Cells	[99]
Microfluidic chip with glass pipettes	Chitosan–alginate	HepG2 cells	[97]
	Alginate (hollow fiber)	HIVE-78	[98]
Capillary tube	Gelatin-HPA	MDCK cells, NIH/3T3 fibroblasts	[141]
Coaxial triple cylinder	Alginate (hollow microfiber)	L929 cells	[142]
PDMS-based microfluidic chip	Alginate	WJMSCs (K562)	[92]
		Hepatocyte, fibroblast (L929)	[90,100]
		3T3, HeLa cells, PC12 cells	[93]
	Collagen-alginate	pancreatic islets	[107]
		Hybrid spheroid of pancreatic islets and hepatocytes	[101]
Coaxial double-orifice spinneret	Alginate derivative possessing phenolic hydroxyl moieties (Alg-Ph)	HeLa cells, fibroblasts (10T1/2 cells)	[143]
Double-coaxial microfluidic device	Alginate, alginate-agaroseIPN hydrogel	Fibroblast, myocyte, endothelial cell, nerve cell, epithelial cell, NIH/3T3-PCol, NIH/3T3-ACol, NIH/3T3-Fib, HepG2-PCol, primary islet cells	[102]

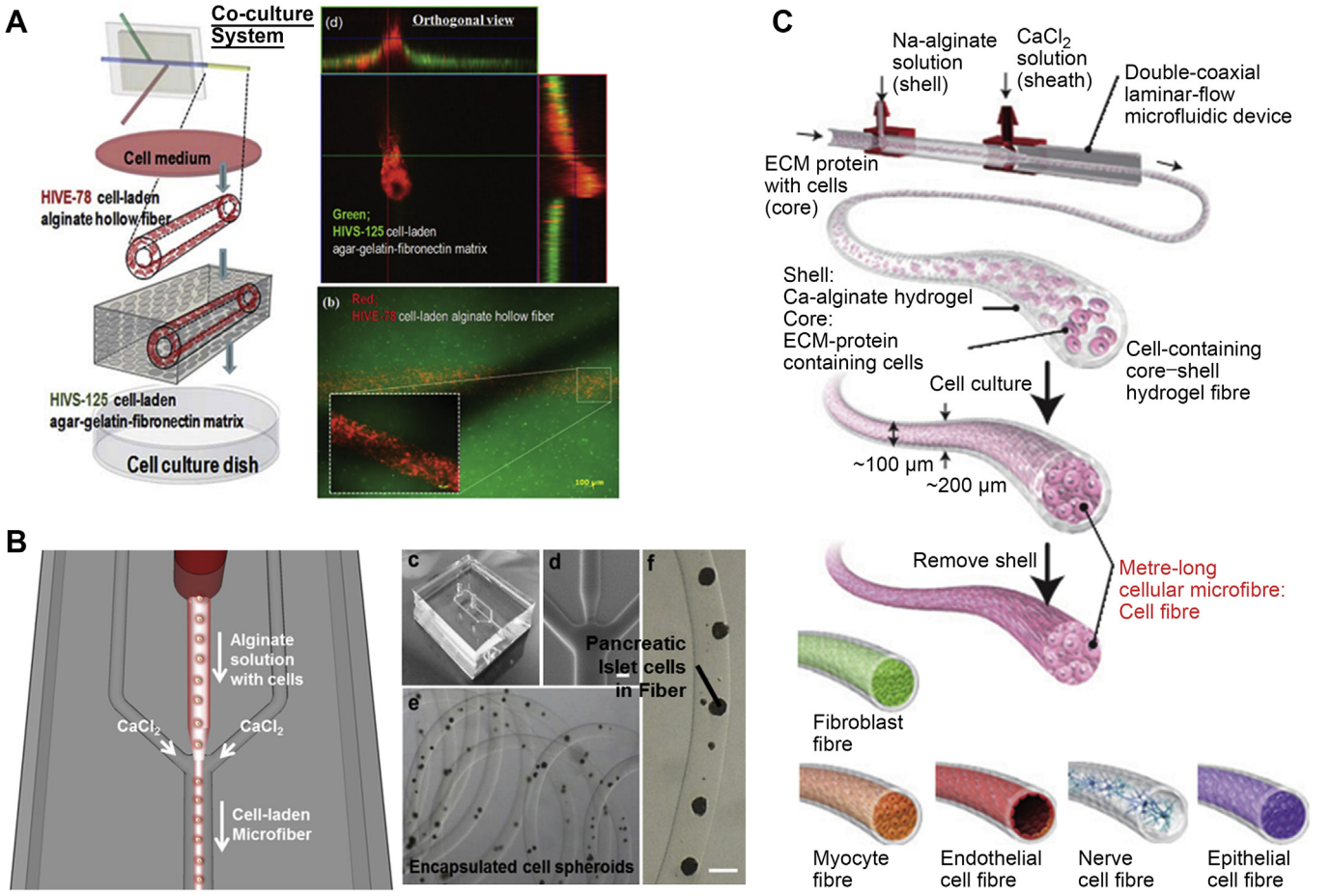

Fig. 4. Cell encapsulation using microfibers containing a variety of cell types. (A) The fabrication of cell-laden alginate hollow microfiber using microfluidic chip with glass pipettes. HIVE-78 cells were encapsulated to mimic the blood vessels that are central to gas and nutrient delivery in tissues. (Lee et al.: Synthesis of cell-laden alginate hollow fibers using microfluidic chips and microvascularized tissue engineering applications. Small. 2009. 5. 1264–1268. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission). (B) A PDMS-based microfluidic chip for three-dimensional co-cultured hybrid spheroids composed of primary islets and hepatocytes in alginate microfiber. (Reprinted from Biomaterials, 34, Jun et al., 3D co-culturing model of primary pancreatic islets and hepatocytes in hybrid spheroid to overcome pancreatic cell shortage, 3784–3794, Copyright 2013, with permission from Elsevier). (C) The fabrication of a meter-long cellular microfiber, cell fiber, by removing hydrogel coating after cell encapsulation by using double-coaxial microfluidic device followed by cell culture with various cell types. (Reprinted by permission from Macmillan Publisher Ltd: Nature Materials [102], copyright 2013).

engineering three-dimensional cultures by coding a variety of cell types and nutrients or gases essential for cell survival. Jun et al. encapsulated primary pancreatic islets [101] and three-dimensional co-cultured hybrid spheroids composed of primary pancreatic islets and hepatocytes [101] in a microfiber (Fig. 4(B)). The pancreatic-like functions of the encapsulated cells were demonstrated in an *in vivo* mouse implantation experiment conducted over one month They emphasized that the three-dimensional co-culturing of hybrid spheroids composed of primary pancreatic islets and hepatocytes offers a solution to the islet cell shortage problem, and that cell encapsulation in microfibers offers a solution to immune rejection after transplantation. Onoe et al. [102] fabricated meter-long cellular microfiber, cell fiber, by removing hydrogel coating after cell encapsulation followed by cell culture with various cell types like fibroblasts, endothelial cells, myocytes, nerve cells, and epithelial cells. They also developed a weaving system that could be used to weave fibers containing different cell types into a three-dimensional structure (Fig. 4(C)).

5.1. Cell-encapsulating microfibers for tissue engineering applications

To date, cell-encapsulated particles have been broadly used in tissue engineering or regeneration of organ function; in contrast, cell-encapsulated fibers were not popularly employed. Recent progress in microfluidic spinning technology enabled the stable encapsulation of cells in the microfibers, and these fibers are extending their applications. Here, we describe their applications in tissue engineering.

5.1.1. Neural system

Most of the tissue engineering approaches relating to neural tissue have attempted to regenerate and reconnect damaged or disconnected central nerves and peripheral nerves and to make them function again [103]. In neural tissue, a signal is transmitted from the axon terminals of presynaptic cells to dendrites in post-synaptic cells. If a neurite is extended along several different directions, the signal transmission can be incorrectly redirected. Thus, neurite alignment is very important in neural tissue engineering efforts having the goal of regenerating nerve tissue and restoring normal function. Many studies have examined neurite alignment for neural tissue engineering purposes. Gomez et al. [104] reported that the physical cues from microchannels and microtopographic features are preferred to chemical cues, such as NGF or laminin, for axon initiation or polarization. Kang et al. [105] reported flat microfibers bearing microgrooved surfaces to provide guiding topographical characteristics along the fiber. The microgrooves could be formed simply by engraving groove on the surface

of microfluidic channel. They observed that neurites extended along the directions of the grooves. In this case, the neuronal cells were only seeded on the surfaces of the fibers, and the cell-aligned fiber could be encapsulated with hydrogel. Onoe et al. [102] encapsulated neuronal cells in alginate fibers to form nerve cell fibers. They observed signal transduction along the nerve cell fibers using Ca^{2+} imaging. Such alignment of neurons will enhance the regeneration of neural system.

5.1.2. Pancreas

The goal of encapsulating pancreatic islets is to reduce the host immune response. The encapsulation of cells using an artificial membrane protects transplanted tissue from the host immune system. Encapsulation of islets can offer a solution to the shortages in donors for clinical transplantation because it allows the clinical use of islets from other species or engineered insulin-producing cells from stem cells [48,52]. The islet spheroids that embedded in collagen-alginate microsheet functioned well in diabetic mice over a 4 week study, so the mice enabled the achievement of normal glucose level [89]. Jun et al. [101] described the development of a fiber-type artificial pancreas model. Primary rat pancreatic islets and hepatocytes were cultured in hybrid 3D spheroids with a uniform size using concave microwells. The hybrid spheroids were encapsulated in alginate microfibers fabricated using a coaxial flow channel method [106], and the insulin secreting functions were evaluated *in vivo* by intraperitoneal xenotransplantation in male Balb/c mice. The mice carrying microfibers consistently maintained a normoglycemia state over 4 weeks. Jun et al. also reported that microfluidics-generated pancreatic islet microfibers enhanced immunoprotection [107]. Onoe et al., demonstrated the encapsulation of pancreatic islet cells in meter-long core–shell hydrogel microfibers. The fiber structures were fabricated using a double-coaxial laminar flow microfluidic device. [102] The fiber cores contained cells and ECM proteins, and the fiber shell was formed from an alginate-agarose hydrogel. The generated fibers were transplanted into the subrenal capsular space in diabetic mice using a microcatheter. The microcatheter was used to precisely fold the fibers and to maintain the contact area between the implanted cell fibers and the renal capsule to promote the efficient secretion of insulin. The blood glucose levels of the recipient mice were normalized within about two weeks.

5.1.3. Liver

The liver plays an important role in the body by detoxifying the blood and secreting several plasma proteins. Hepatocytes provide an enormous portion of the liver functions. Hepatocyte-based cell therapies have been studied in an effort to identify alternatives to liver transplantation. Although hepatocytes display a high regeneration capacity *in vivo*, the viability of most primary hepatocytes after isolation tends to be poor. The maintenance and improvement of hepatocyte function *in vitro* for clinical hepatocyte transplantation applications remains challenging. The three-dimensional culturing of hepatocytes has been examined as a possible solution. Lee et al. used wet spinning fiber fabrication methods based on chitosan–alginate to encapsulate HepG2 cells [97]. They described the fabrication of chitosan–alginate fibers using a coaxial flow microfluidic chip, and the cell behavior and viability were tested. Park et al. [90] fabricated a fibrous scaffold composed of hepatocyte-embedded fibers with microfluidic chip. They showed the fibrous scaffold enables better cell viability by providing high diffusion capability of molecules in massive cell culture. Kang et al. [100] and Yamada et al. [108] developed an alginate-based cell cultivation fiber with controlled cellular organization. Hepatocytes were sandwiched between fibroblast cells along the length of the fiber to mimic the hepatic cord structures found in the liver. We have *in situ* encapsulated hepatocyte spheroids co-cultured with sinus endothelial cells and hepatic stellate cells and implanted them in to the abdomen cavity of mouse with 90% hepatotectomy. As result, most of mice were survived and any immune attraction was not observed (results are not published yet) indicating that encapsulated hepatic cells can support the liver function for a certain period.

5.1.4. Microscale blood vessels

Cardiovascular disease remains the number one cause of death worldwide. [109] Atherosclerotic plaques can result in severe occlusions of peripheral and coronary arteries. For this reason, the development of artificial blood vessels that could replace damaged blood vessels is an active area of research. One of the big challenge is to engineer microscale vessels avoiding blood clots. A few research groups have attempted to fabricate artificial blood vessels using microtechnology to deliver blood into the engineered tissue. Because blood vessels are tubular in structure, researchers have used microfibers for artificial blood vessel formation. Lee et al. [98] fabricated HIVE-78 cell-laden alginate microscale hollow fibers using microfluidic chip. Onoe et al. [102] used a double-coaxial microfluidic device to form a tubular structure bearing a monolayer of primary human umbilical vein endothelial cells (HUVECs) along the direction of the fiber after 4 days of culturing.

6. Challenges and future directions for cell encapsulation using microsystems

In this review, we have surveyed several effective and safe cell encapsulation methods based on microtechnology and their potential applications in tissue engineering. Several crucial problems deserve attention before these methods may be implemented in clinical applications. Fig. 5 demonstrates the scheme of cell encapsulation for cell therapy application. Various cell types from xeno or allo species or stem cells could be used as a cell source, and they are scaled up and encapsulated enough to replace or support the function of failed organ. Finally, they are implanted into the body to regenerate organ functions. Although microscale cellular encapsulation approaches enable the precise control over cell size and shape, unlike conventional bioreactors, mass production via cost-effective and labor-efficient methods remains a great challenging. The scale-up of microtechnology-based production systems and the automation for the production of well-controlled encapsulation products are critical issues. For example, to replace the liver functions, a number of cells ($\sim 10^{10}$ hepatocytes) are required [110,111]. Here, the encapsulation of such a large quantity of cells within one day poses a significant challenge. These problems may be addressed by combining large numbers of microwell-based bioreactors using an electro-mechanical automation system. Another aspect of production that requires attention is the consistent control over the thickness and porosity of capsules to enhance diffusion of nutrients and gases and the secretion of wastes. Recent progress in microfluidic spinning or particle generation could dramatically enhance the precision with which the fiber thickness is controlled without the need for complicated devices or process. The surface tension between immiscible liquids in a microfluidic channel can drive cells to form uniformly self-encapsulated structures within a hydrogel. Microfluidic spinning enables the production of self-assembled meter-long thread-shaped tissues, and the technique is very attractive for engineering bioartificial muscles and neural systems which is difficult by conventional method. From our experience, the cell encapsulation in the fiber has many advantages in the easy formation of any shape of 3D tissues with high diffusion of nutrient and oxygen and the simple handling of encapsulated cells. The cell-encapsulated fiber

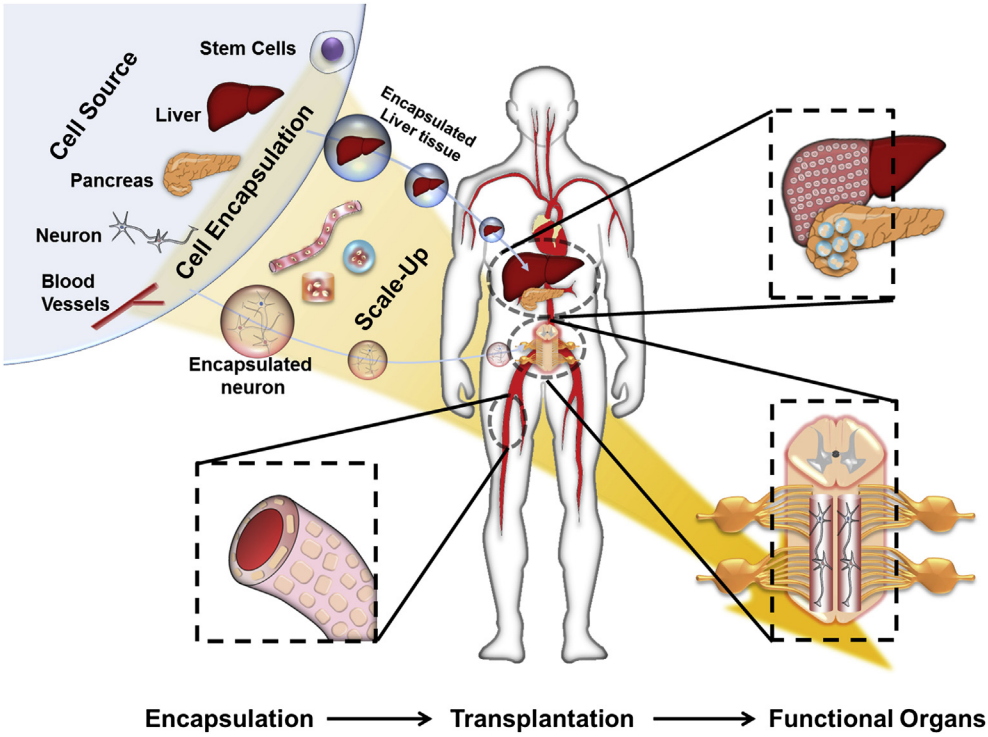

Fig. 5. Schematic diagram of cell encapsulation for cell therapy application. Various cell types can be used as a cell source, and they are encapsulated and scale up to be a functional organ and are implanted into the body.

can also be injected through syringe needle, which is one of critical advantages in maintaining injected cells stably inside the body. Although encapsulation of cells in microfiber has been successful, the mechanical weakness and rapid biodegradation of microfibers poses an ongoing technical challenge. The employment of diverse materials for encapsulation could resolve many of these problems. Newly developed microtechnology-based encapsulation methods could protect cells, enhance cell viability and performance by precisely controlling the thickness and porosity of encapsulating membrane compared to conventional methods, thereby enabling the extension of these applications to a broad range of organ-function regeneration.

7. Conclusions

Cells from another individual or species can induce an immune response when implanted into a new host, resulting in the functional loss of cells and implant failure. In an effort to prevent the initiation of a host immune response, a number of cell encapsulation approaches have been developed over the past few decades. Conventional encapsulation methods generally do not permit significant control over the capsule size and thickness, and achieving uniform cell distributions remains challenging. Recent advances in microtechnology-based cell encapsulation methods could go some way toward resolving the problems associated with conventional encapsulation problems, cell viability, and cell function, in implanted cells. Despite these advantages, several impediments to clinical applications remain. Biocompatibility, biodegradability, the maintenance of long-term implants, control over the thickness and porosity of capsules to achieve enhanced diffusion, and mass production via cost-effective and labor-efficient methods, continue to pose significant challenges. The rapid progress in the technologies used for microfabrication and biomaterial development could resolve these problems and promises to further advance cell encapsulation technologies.

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