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Review Article

Microdroplet-based Cell Culture Models and Their Application

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Abstract Microfluidic systems offer precisely controlled experimental conditions for efficient study of mammalian cells, ranged from a single cell to matrix-Based three-dimensional (3D) *in vitro* culture models. Droplet-based microfluidic system is a robust and highly reproducible device enabling encapsulation of a cell or cells within well-confined microenvironment. Recently, such droplet-based cell culture models have drawn much attention due to their unique properties such that conventional culture systems couldn't provide. The encapsulation of cells in specifically designed aqueous phase of a microfluidic system can provide profound understand of cell to cell and cell to extracellular matrix interactions, also can be used to regulate various cell behaviors. A droplet-based cell culture system allows better control over confinement for culturing, maintaining, and analyzing cells, such as high-throughput screening. In this review, we discuss recent researches on microdroplet-based 3D cell culture models, and advanced applications of microfluidic systems.

Keywords: Microdroplet, 3D cell culture, High-throughput, Single cell analysis, Multicellular spheroid, Cell-laden ECM

Introduction

Droplet microfluidics has gained much attention for large-scale drug research and screening as it offers the convenience of high-throughput, requiring only a few microliters of a sample and a small number of cells[1-3]. Microfluidic droplets containing just a few picoliters (pLs) to nanoliters (nLs) can be generated at high frequencies (Hz-kHz), simply by pressure-driven flow. Highly monodisperse aqueous droplets are surrounded by immiscible inert oil within microfluidic channels, such that each droplet acts as a reactor. Aqueous phases containing biological samples, such as cells, nucleic acids (DNA or RNA), bacteria, and small molecules can be encapsulated into microdroplets[4,5]. Encapsulated biomolecules in microdroplets are useful in various applications, such as high-throughput single-cell analysis, and complex biochemical assays within well-confined 3D cell cultures[6].

In this review, we focus on emerging researches in 3D cell culture using droplet-based microfluidics, classified into single-cell, multicellular spheroid, and extracellular matrix (ECM) microbead-based 3D cell culture models. This review article offers an overview of the methods and applications of microdroplet-based 3D cell culture models.

Microdroplet-based Cell Culture Model

Figure 1 shows various microdroplet-based 3D cell culture models, the details of which are described in the following sections as they relate to the microdroplet-based cell culture platform and its applications, single cell analysis, multicellular spheroids, and cell-laden ECM microbead. Table 1 recognizes microdroplet-based cell culture systems by encapsulation materials and encapsulated cell type, and their applications. Representative research studies with each cell culture system are summarized in Table 1.

Single Cell Encapsulation

Typically, the cells are randomly distributed in an aque-

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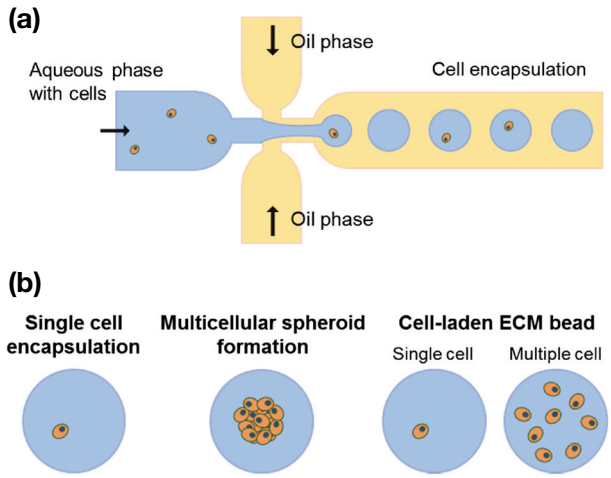

Figure 1. Microdroplet-based cell culture platforms. (a) Schematic of microfluidic based droplet formation for 3D cell culture model. (b) Classification of microdroplet-based 3D cell culture model, single cell encapsulation, multicellular spheroid formation, and cell-laden ECM bead.

ous phase and randomly encapsulated within microdroplets according to a Poisson distribution effect (only 3.84% of all droplets contain a single cell). To overcome this limitation in conventional droplet based techniques, Edd *et al*. developed an alternating orientation of the cells within the microfluidics to allow ordered single-cell encapsulation with 97% efficiency[22].

Compartmentalization of single cells is a representative feature of the droplet microfluidics system, in which each microdroplet acts as an isolated microsystem[9,11,23]. Cells and detection probes can be encapsulated within the microdroplets for detection and sorting processes. Secreted biomolecules from such compartmentalized single cell can easily reach detectable level inside of the microdroplet, enabling the rapid detection of microdroplets containing target cells by fluorescence-activated cell sorting (FACS: Figure 2a)[24]. For example, Mazutis *et al*. demonstrated microdroplet-based high-throughput analysis of a single mouse hybridoma cell by using a FACS system[9]. The secreted antibodies from single cells can react with co-encapsulated fluorescent probe and antibody coated-beads within microdroplets. The fluorescence signal can be detected and sorted at ~200 Hz through a FACS system[9]. Moreover, Brouzes *et al*. developed a microdroplet-based cytotoxicity screening platform for an optically-coded drug library[11].

Intracellular biomolecules in encapsulated cells can also be assayed through lysing the cells[25]. These lysed biomolecules can be analyzed biochemically and genetically; for example, DNA or RNA can be amplified within the droplets[7,26-28]. Recently, many researchers have studied single-cell encapsulation approaches to improve analysis of intracellular nucleic acids in target cells. Small amounts of nucleic acid can be detected readily after amplification through the polymerase chain reaction (PCR) in microdroplets (Figure 2b). Eastburn *et al*. identified and analyzed amplified nucleic acids, particularly RNA, from single prostate cancer cells within microdroplets using PCR-activated cell sorting (PACS) technology. Their PACS system allowed simultaneous multiplexed reactions to occur, with each reaction representing amplification from a single cell[7].

More recently, the encapsulation of single cells in microdroplets has provided a powerful tool to study single-cell heterogeneity. The cellular phenotype, represented by varying levels of molecular markers, such as genes and proteins, determines the unique functions and activities of cells, such as cellular communication, and metabolism[8,23,29]. The analysis of molecular biomarkers in cell populations, as typically used, can be confounded by inherent cellular heterogeneity, restricting to distinguish the differences between individual cells. However, with droplet microfluidics, it may be possible to better understand cellular heterogeneity with a well-integrated isolating, culturing, and analyzing system. Thus, recently, several research groups have described droplet-based microfluidic platforms for high-throughput analysis and screening of cellular heterogeneity at a single-cell level[9,11,29].

Such microdroplet-based single-cell analysis are expected to have a broad impact on diverse biological platforms, including single-cell phenotyping and biomarker discovery for diagnostics and therapeutics[8,10].

Multicellular Spheroid Culture

Multicellular spheroid cultures are commonly used as 3D cell culture models to better mimic the *in vivo* microenvironment with cell-cell interactions, which is lost in two-dimensional (2D) monolayer cultures[16,17,30-32]. Cell-cell interactions regulate many biological functions, such as cell proliferation, viability, and phenotype, through contact-dependent (juxtacrine) signaling, mediated by direct cell-cell communication between neighboring cells[33,34]. Compared with conventional 2D monolayer cultures, the multicellular spheroid provides a unique microenvironment with cell-cell interactions. Due to its higher similarity to *in vivo* tissue, the multicellular spheroid provides a valuable tool for more predictive biomedical research[15,33,35-37].

Traditional methods used to form multicellular spher-

Table 1. Overview of microdroplet-based 3D cell culture model.

	Encapsulation material	Cell line	Application	Year	Ref.
Single cell analysis	Lysis buffer	Human prostate cancer cell (DU145) B-lymphocyte cell line (Raji)	PCR-activated cell sorting DNA sequencing	2014	7
	Alginate	Hybridoma cells producing Anti-myc and anti-TNFα antibodies	Heterogeneous immunoassays Screening antigen-specific antibody secreting cells FACS	2014	8
	Culture media Streptavidin-coated beads coated capture antibody Detection antibody	Mouse hybridoma cells (9E10) Human leukemia cells (K562)	High throughput screening of specific antibody secreted cell FACS	2013	9
	Cell suspension Antibody-conjugated beads	Immune dendritic cells	Monitoring live single cell surface markers Antibody secretion analysis	2013	10
	Culture media Drug	Human monocytic U937 cells	Screening drug library	2009	11
	Alginate	Human ovary carcinoma cell line (HeLa)	FACS	2013	12
Multicellular spheroids	Alginate	Human breast cancer cells (MCF-7) Bone marrow fibroblast line (HS-5)	Co-encapsulation of tumor cells and fibroblasts Anticancer drug screening	2016	13
	Culture media	Human glioblastoma cell line (UVW)	Assessment of radiation (X-ray) toxicity	2016	14
	Alginate Magnetic particles	Human ovary carcinoma cell line (HeLa)	Magnetically separation system of multicellular spheroids Drug screening	2013	15
	Alginate	Human mesenchymal stem cell (hMSC)	Double emulsion (w/o/w) droplet formation Stem cell differentiation	2013	16
	Alginate	Breast cancer cells (LCC6/Her2)	Anticancer drug testing Microseive-based microdroplet trapping	2010	17
	Alginate	Mouse embryonic carcinoma (EC) cell line (P19 EC)	Cell viability test	2015	18
Cell-laden ECM	Alginate–Matrigel mixed solutions	Human ovary carcinoma cell line (HeLa)	Anticancer drug testing	2014	19
	Collagen	Breast cancer cell (MDA-MB-231)	High-throughput cell migration study	2016	20
	Collagen-gelatin mixed solutions	Mouse 3T3 fibroblast	Cellular viability Analysis of cell spreading	2013	21
	Collagen	Gastric cancer cell lines (AGS, Hs746T)	Analysis of cellular morphology Drug resistance test	Unpublished	

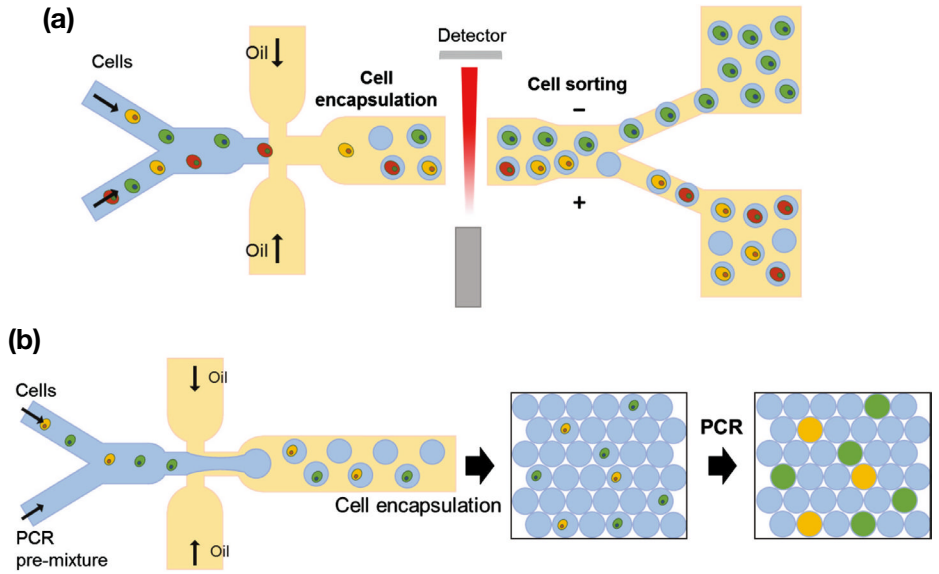

Figure 2. Microfluidic droplet based single-cell analysis. (a) Droplet-based microfluidics for single cell analysis and sorting by fluorescence-activated cell sorting (FACS) technique. (b) Detection and identification of nucleic acids in single cell by amplifying through polymerase chain reaction (PCR) in microdroplets.

oids include floating, hanging drop, gyratory rotation, and liquid overlay culture techniques[14,36]. While those methods are commonly used in research laboratories, they are labor intensive, have a low-yield, and lead to size heterogeneity in resulting formations. To address these drawbacks, microfluidic droplet formation technologies have recently been developed as tools for size-controllable formation and culturing of multicellular spheroids. To date, droplet-based multicellular spheroids formation have been developed by encapsulating and culturing cell suspensions within non-adherent hydrogels, such as alginate, agarose, gelatin, and polyethylene glycol (PEG), and their derivatives. Encapsulated cells in non-adherent confinement aggregate spontaneously and form multicellular spheroids.

Previous research has indicated that multicellular spheroids enhance differentiation to specific cell types[16,38-40]. In particular, Chan *et al*. demonstrated the water-in-oil-in-water (w/o/w) double emulsion droplets for multicellular stem cell spheroids to enhance osteogenic differentiation (Figure 3a)[16]. The encapsulated human mesenchymal stem cells (hMSC) showed enhanced differentiation in alginate microdroplets.

Spheroids have also been used to examine and analyze the cytotoxic activity of anticancer drugs and radiation treatment[13,14]. Sabhachandani *et al*. fabricated cell-laden alginate microbeads as 3D multicellular tumor spheroids, and used them as a more effective preclinical drug-resistance screening model[13]. Recently, McMillan *et al*. developed the formation of emulsion-based multicellular tumor spheroids with long-term culture, and analyzed the effects of radiotherapy on multicellular cancer spheroids, depending on their size and radiation dose[14].

Despite its many advantages, however, the technique still has difficulties in forming uniformly sized cellular spheroids, due to the difficulty in regulating the number of encapsulated cells within non-adherent microdroplets. To overcome this issue, Kim *et al*. introduced an advanced cell-scattering step in a droplet-based microfluidic device to make more uniform spheroids by dispersing cell clusters (Figure 3b)[18].

It has also been difficult to effectively collect and separate spheroids from toxic oil in microfluidic device. Yoon *et al*. developed a magnetically separable alginate bead inside of microfluidic channel (Figure 3c)[15]. Cells and magnetic nanoparticles were encapsulated within alginate beads, which were uniformly generated in the oil phase. By applying an external magnetic force, alginate beads in laminar flows were magnetically transferred into the culture medium phase from the oil phase. Thus, microfluidic chip enhanced the formation and collection of spheroids from the oil phase.

Cell-laden ECM Bead

Most cells in the human body are connected to their neighboring cells via the ECM allowing interaction and communication[35,41,42]. Many researchers have pro-

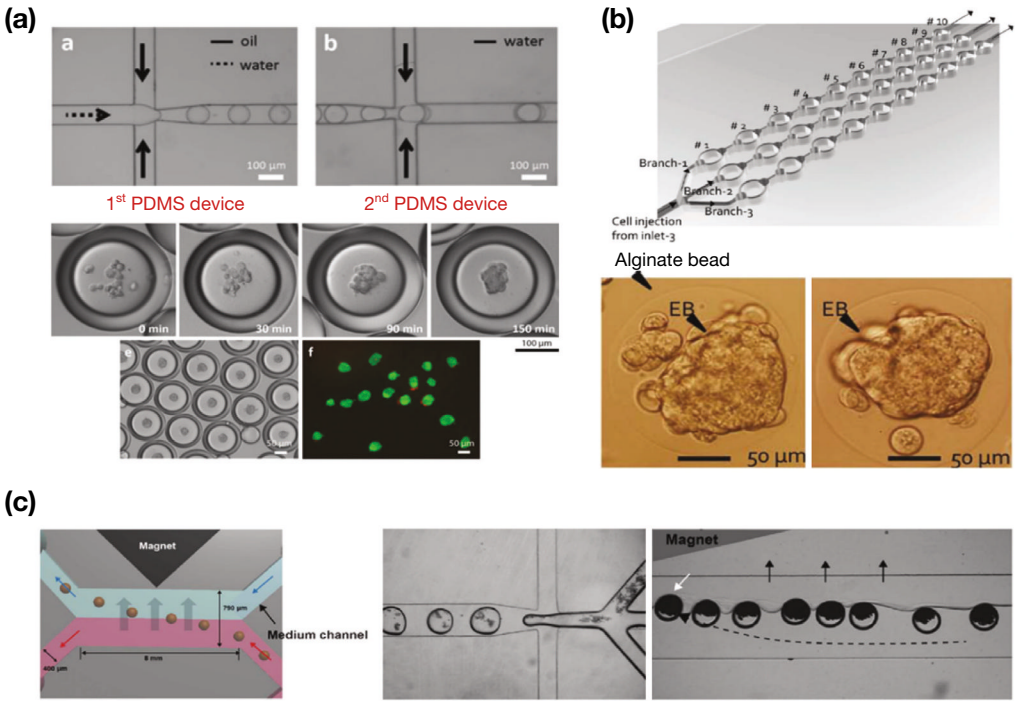

Figure 3. Formation of multicellular spheroids in microdroplets. (a) Multicellular spheroid formation in alginate-based micrdroplet by w/o/w microfluidic system. Images reproduced from ref.[16] with permission. Copyright 2013, Nature Publishing Group. (b) Cell-scattering encapsulation device for forming uniform-sized embryoid bodies. Images reproduced from ref.[18] with permission. Copyright 2015, The Korean BioChip Society and Springer-Verlag Berlin Heidelberg. (c) Retrieval of multicellular spheroid by magnetic nanoparticle in single microdevice. Images reproduced from ref.[15] with permission. Copyright 2013, The Royal Society of Chemistry.

moted 3D platforms for studying interactions in the cellular microenvironment to identify the key factors involved in migration mode and cellular responses regulation[12,20,43]. Indeed, the cellular behavior and function are influenced greatly by the mechanical and chemical properties of the ECM microenvironment[44-46]. Therefore, the studying 3D cellular behavior has gained attention, as it is increasingly able to better mimic the *in vivo* microenvironment.

A droplet microfluidic devices has been developed to generate cell-encapsulated ECM mirobeads[2,3]. As a valuable 3D model for the high-throughput evaluation of drug, a cell-laden ECM can provide a more comprehensive assessment of therapeutic strategies, and enables the study of ECM-related microenvironments. ECM-based 3D microbead can provide cell-cell communications and cell-ECM interactions[34,47-49].

To investigate the effects of cell-ECM interactions in cell-laden ECM microbeads, Wang *et al*. used an alginate-Matrigel mixed hydrogel to form multicellular spheroids within a cell-interactive matrix; these cell-laden ECM beads were then used in anticancer drug testing (Figure 4a)[19]. The multicellular tumor spheroids formed simultaneously with uniform morphology and were closely connected to each other.

Various cell-interactive hydrogels have been used for cell-encapsulation to control cell-ECM interactions. It is important to control the mechanical properties of the hydrogels such as stiffness, network architectures, and porosity. Many examples for successful encapsulation of cells into a hydrogel have been reported. Natural ECM materials, such as collagen and fibrin, provide superior cell binding and motility, but often lack mechanical stability[21,50]. In contrast, synthetic polymeric hydrogels have an advantage over natural ones in that their mechanical properties can be controlled readily, but additional processes are often needed to obtaining cell-binding properties[51].

Ma *et al*. used a gelatin-collagen mixed hydrogel to form monodisperse microbeads with a tissue-relevant range of stiffness (1-10 kPa), crosslinked by riboflavin and blue light irradiation[21]. In contrast, Che *et al*. and Jang *et al*. fabricated cell-laden collagen microbead through the polymerization of a collagen solution after generating the mechanically stable microbeads (Figure 4b and 4c). In Che *et al*.'s study[20], breast can-

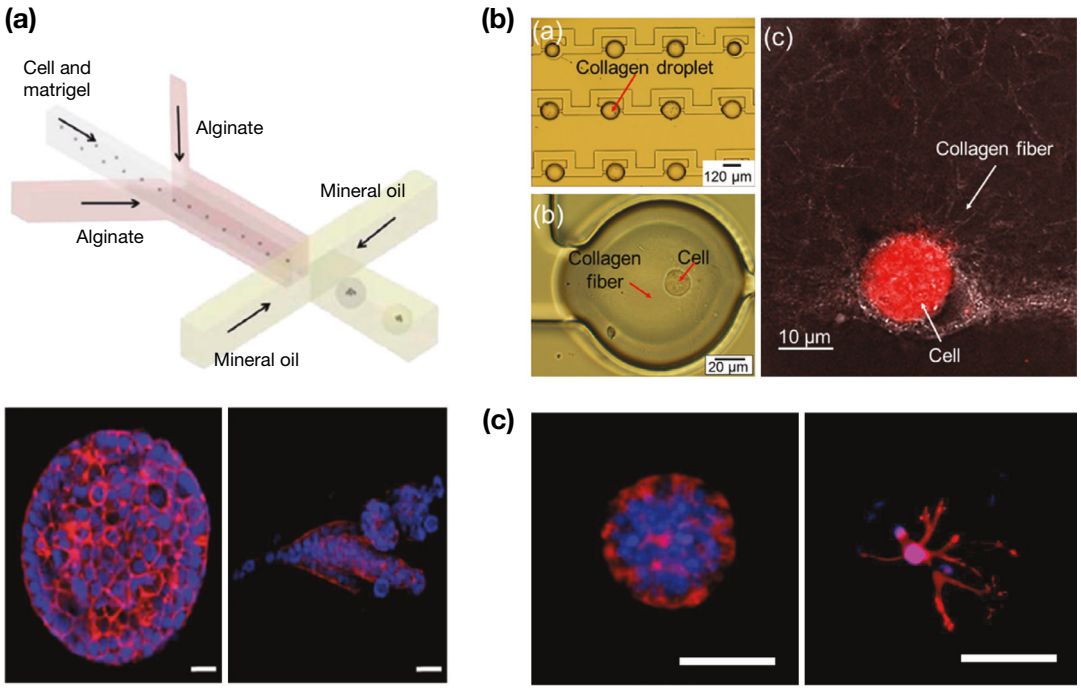

Figure 4. Formation of cell-laden ECM-based microdroplet for cell culture. (a) Schematic of microfluidic cells encapsulation in alginate-Matrigel mixed droplets. Images reproduced from ref.[19] with permission. Copyright 2014, The Royal Society of Chemistry. (b) Collagen based microdroplet for analysis cell migration. Images reproduced from ref.[20]. Copyright 2016, MDPI. (c) Analysis of cellular morphology and drug resistance in ECM microbead (Jang *et al*., unpublished data).

cer cell-laden collagen microdroplets generated and arrayed in microchambers on the integrated device (Figure 4b). They observed polymerized collagen fibers interacting with cells using confocal reflectance microscopy. Using arrayed cell-laden ECM beads, the migration trajectory of the cells has been recorded via optical microscopy. Recently, we generated a cell-laden collagen microbead-based gastric cancer model in an analysis of drug resistance. Using the microtumor model, we demonstrated the difference in cellular functional characteristics and drug resistance depending on the gastric cancer cell types (Figure 4c, unpublished data). Such cell-laden ECM bead-based 3D models can potentially be used to study cancer cell-ECM interactions and ECM remodeling in a more *in vivo* tumor microenvironments.

Conclusions and Perspectives

In summary, we have provided an overview of recent developments in droplet-based cell culture systems in microtissue engineering and biomedical applications. The use of microfluidic-based droplet techniques enables scalable assays with single cells, multicellular spheroids, and cell-laden microtissues. In the near future, we expect that droplet-based microscale cell culture systems will be used as cost-effective and highly reproducible models for drug discovery and development.

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