New materials for microfluidics in biology
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With its continuous progress, microfluidics has become a key enabling technology in biological research. During the past few years, the major growth of microfluidics shifted to the introduction of new materials in making microfluidic chips, primarily driven by the demand of versatile strategies to interface microfluidics with biological cell studies. Although polydimethylsiloxane is still used as primary frame material, hydrogels have been increasingly employed in cell-culture related applications. Moreover, plastics and paper are attracting more attention in commercial device fabrication. Aiming to reflect this trend, current review focuses on the progress of microfluidic chip materials over the time span of January 2011 through June 2013, and provides critical discussion of the resulting major new tools in biological research.

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Introduction
Microfluidics generally refers to technologies using microfabricated structures to precisely manipulate fluids at nanoliter to femtoliter scale [1]. Operating at the scale close to the sizes of biological cells, microfluidic technologies exhibit unique power to realize many valuable functions hard or impossible to achieve using conventional biological technologies [2,3]. With microfluidic technologies, different cell strains could be precisely patterned and co-cultured, mimicking the organization of tissues or organs [4,5,6]. The microenvironment around the cells in a microchannel could be well controlled, utilizing the low-dead-volume fluid manipulation strategies and unique hydrodynamic properties, for example, laminar flow in a microfluidic channel [3]. Besides, the scale at which microfluidics operates inherently fits the need of biological analysis, not only because it dramatically reduces the consumption of expensive bioreagents but more importantly it could restrict the diffusion and dilution of the content released from a few or even single cells, therefore well adaptable to biochemical analyses at the single cell level [7]. Finally, the microfluidic channels could further integrate with other microcomponents, for example, integrated electrodes, to achieve localized controlling or sensing [8,9].

During the past few years, the major growth of microfluidics shifted to the introduction of new materials in making microfluidic chips, primarily driven by the demand of versatile strategies to interface microfluidics with biological cell studies [6,10]. With its continuous progress, microfluidics has become a key enabling technology in biological research. Aiming to reflect this trend, current review focuses on the progress of microfluidic chip materials over the time span of January 2011 through June 2013.

Elastomer
Microfluidics debuted with devices fabricated in silicon and glass. These materials exhibit outstanding inertness, excellent strength and thermoconductivity, allowing solvent involved processes and high speed capillary electrophoresis [11]. However, microchannels made of these materials are impermeable to gases thus unable to support long term cell culture.

Introduced after silicon and glass, silicone-based elastomers, primarily, polydimethylsiloxane (PDMS), have become the fundamental material for microfluidic chip fabrication [12]. PDMS is highly permeable to gases and the best material to fabricate integrated valves; many milestone demonstrations have proven its great role in cell-related microfluidics [7]. Although hydrogels are increasingly employed in cell-related microfluidic applications, the elasticity, better optical and mechanical property of PDMS makes it irreplaceable as the frame material; thus PDMS continues to be the most commonly used material for this purpose. Specifically, PDMS has been frequently employed to generate scaffolding channel structure for in situ fabrication of incorporated hydrogel structures. Related applications in on-chip cell-culture based studies are presented in the section of hydrogels.

For various demands of culture-related microfluidic tools, new implementations of PDMS device are continuously being explored. The elasticity has been the key attraction of PDMS. One major implementation is integrated pneumatic valves. The highest density of integration exceeded 1 million pneumatic valves in PDMS per square centimeter [13]. This enables very large scale integration of...
multiple processing elements for high throughput processing in parallel, which could be very useful in single cell array analyses and bio/pre-drug screening. PDMS valve arrays were also employed for programmable fabrication of composite materials. Lee et al. used this strategy to fabricate cell-laden microfibers, in which the composition of a certain region of the fiber was coded by the array of pneumatic valves during the laminar-flow assisted fabrication (Figure 1A) [14*].

The elasticity of PDMS was also utilized in other new approaches, primarily, in forming a deformable frame in a hybrid structure. One excellent example is the work of Ingber and his colleagues, which utilized PDMS as skeleton of an elastic hybrid membrane in a delicate device design to mimic the function of a lung [15**]. In their device, a porous PDMS thin membrane covered with cell-laden hydrogel was used to separate two chambers, mimicking the interface of lung; the membrane could be stretched by pneumatically activated deformable PDMS chambers connected to each end of the membrane (Figure 1B). In this way, the device successfully reconstructed the function of lung. Another good example is the work of Jiang group in forming a 3D structure out of a hybrid biomembrane. They coated a pre-stretched PDMS membrane with a cell-laden hydrogel layer; once the stress was released, the hybrid membrane contracted and rolled spontaneously, generating a tubular structure like blood vessels (Figure 1C) [16].

**Plastics**

Plastic microfluidic devices are increasingly used for developing biological assays, particularly for commercial implements [17,18]. Plastics became practical microfluidic chip material after the development of a convenient microfabrication strategy, primarily, soft lithography. In general, plastics are still less commonly used in research laboratories for prototypical device

Figure 1

(a) Digitally tunable physicochemical coding of material composition and topography in continuous microfibers using a PDMS Device containing microvalve array. (b) Reconstituting organ-level lung functions on a PDMS-hydrogel hybrid chip. (c) A strategy for depositing different types of cells in three dimensions to mimic tubular structures in tissues.
fabrication. One possible reason is that their microfabrication is normally based on thermoprocessing, which is more suitable for mass production other than prototyping, compared to the protocol for PDMS device fabrication [10]. However, it is worth noting that aside of microfluidics-based studies, most biological researches have been based on devices made of plastics, for example, polystyrene and Teflon plastics. Knowledge and experience accumulated with devices made in these plastics are more convenient to be transferred to microfluidic devices fabricated in the same material [19]. Also, devices fabricated in plastics through thermal processing are cheaper than PDMS devices. Therefore, plastic devices show a promising potential and has drawn significant interest in commercial device fabrication: several platforms have been established, for example, for diagnostics of infectious diseases in the developing world [20], and polyester chips for stem cell study [21]. Beebe group reported a rapid prototyping method for arrayed microfluidic systems in polystyrene for cell-based assays [22]; Mathies group demonstrated a disposable roll-to-roll hot embossed electrophoresis chip in PMMA for detection of antibiotic resistance gene mecA in bacteria [23]. The enhanced autofluorescence and impact on cell microscopy for microfabricated thermoplastic (polystyrene and cycloolefin polymer) devices has also been studied [24].

Figure 2

(a) Whole-tetflon microfluidic chips. (b) Directing osteogenesis of stem cells with drug-laden, polymer-microsphere-based micropatterns generated by teflon microfluidic chips.
Teflon, a very special family of plastics, is extremely inert, solvent resistant, antifouling and non-stick, thus broadly used in various fields — examples of biological applications include high precision assay, super clean tools, and biological implants. The lack of efficient microfabrication method had been a hurdle of making microfluidic devices in Teflon materials. Recently, a method based on transfer molding and fusion bonding has been developed to fabricate microfluidic chips in Teflon plastics, which enables the application of this family of plastics in biofluidics (Figure 2A) [25]. Teflon microfluidic chip retains all the superior features the bulk material possesses; in addition, it supports in-channel cell culture, and does not have the problem of channel fouling as PDMS. Moreover, microfabricated Teflon is an excellent template for casting or molding other materials, e.g., cell- or drug-laden hydrogel, owing to its outstanding inertness and non-stickiness (Figure 2B) [26,27].

Hydrogel
Different from other polymers, hydrogels possess macro-molecular structures similar to that of the extracellular matrix (ECM) of biological cells [6]. With water up to over 90% of the total mass, hydrogels are highly porous, allowing diffusion of molecules through the bulk [4]. The inherent cytocompatibility and high permeability make
hydrogels excellent material for encapsulating living cells for experiment [5,7,28]. However, it has been a challenge for conventional tissue engineering to achieve 3D cell culture in hydrogel, owing to the fact that cell-laden hydrogel (or their biological counterpart, multilayer cells embedded in ECM) would not support deep penetration of nutrition and oxygen which restricts the thickness of cell-laden hydrogel within ~500 µm [4]. In recent years, microfluidic technology explored two strategies to overcome this barrier. In the ‘top-down’ strategy, microchannels are fabricated inside the cell-laden hydrogel matrix and function as vascular structures to realize exchange of liquid through the bulk hydrogel. In the ‘bottom-up’ strategy, cell-laden hydrogel is fabricated into particles, fibers or strips, with the assist of microfluidic technology; these elements form gaps when packed up, allowing exchange of molecules through the suspension liquid.

The ‘top-down’ strategy is more straightforward to mimic native tissues, as it directly generates vascular structures in cell-laden hydrogel. The microchannel could be fabricated either by a casting-bonding strategy, or by direct writing strategy. Both natural and synthetic hydrogels have been used for this purpose. One recent example is the work of Jiang group, which realized cell-seeded vascular network to mimic the function of a nephron [29]. In their work, a hydrogel microfluidic chip was fabricated with collagen and alginate through fibril bonding and loaded with epithelial cells and endothelial cells into two adjunct microchannels, respectively (Figure 3A). With the help of the anchoring factor from collagen, the seeded cells attached onto the channel wall, forming vascular structures mimicking the function of a nephron. Alternatively, the device of a ‘top-down’ design could be fabricated in other material, primarily, PDMS, with in situ generated hydrogel structures as cell-laden implanted components. Kamm, Chung and their colleagues reported a versatile platform based on this concept to mimic the interaction of multiple cell types in an in vivo environment [30**]. In their design, parallel microchannels were fabricated in a PDMS device; plugs made of ECM material (collagen) were fabricated in between of each adjacent channels, serving as tunnels that allow diffusive exchange between the two channels (Figure 3B). Also, these hydrogel plugs support attachment of cells to their surfaces and allow other cell types to grow inside. Moreover, diffusion gradient could establish in the hydrogel plugs. In this way, the platform allows study of 3D cell culture in a biochemical gradient environment as well as cell–cell interaction within micrometer scale. Kamm demonstrated the use of similar design in the study of tumor cell intravasation and endothelial barrier function [31]. West and his colleagues developed another approach to generate diffusive barrier in a microfluidic channel, in which a cell-laden PEG hydrogel plug is formed before sealing the channel [32]. Besides, Beebe et al. reported a method utilizing surface adhesion force to generate endothelial-lined microvessels in a hydrogel-filled PDMS microfluidic channel [33].

Another frequently employed strategy forms a hydrogel membrane at the interface of two laminar flow streams with cells attached on or embedded in the membrane (Figure 3C) [34]. Compared to Kamm et al.’s design, this strategy allows even shorter distance between co-cultured cells, and is increasingly adapted in cell–cell interaction studies. Alternatively, as discussed in the section of Elastomers, cell-laden hydrogels could be fabricated into patterned fibers [14*] or stripes [35**], with the help of a programmable laminar flow generator. Also, biomolecules were immobilized on the surface of hydrogel with the assist of gradient generating device and used for quantitative study of stem cells [36].

Besides, there are also non-conventional strategies reported recently for generating hydrogel channel networks. Langer group fabricated 3D interconnected microchannel network in gelatin using sacrificial shellac microfibers, which opens up an inexpensive and easy access way to build 3D vascular network in hydrogels [37]. Khademhosseini and his team developed a method based on sequential assembly of cell-laden hydrogel structures to engineer vascular-like microchannels (Figure 3D) [38].

Also made of hydrogels, cell-encapsulated microbeads generated in microfluidic devices are also increasingly employed in biology research. Compared with channel structures made of hydrogels, cell-encapsulated microbeads are more frequently used in single-cell screening in a high throughput fashion, although there have also been various demonstrations showing strategies for co-encapsulating multiple cell types [39*]. Depending on whether a liquid core is preferred the microgel could be fabricated into solid or hollow beads. A significant advantage of hydrogel microbeads is that by tuning the formula for generating the beads they offer controllable permeability to molecules up to a certain size, while retain larger molecules and particles well restricted in the core [40]. This is beneficial to many analytical processes, e.g., they could serve as isolated reaction chambers allowing parallel operations on thousands of beads in a bulk suspension, while after the reaction those beads with certain probe activated could be picked up using conventional or on-chip flow cytometry for further analysis [41**,42].

**Paper**

Compared to the aforementioned, paper is the most recently introduced material for microfluidic chip fabrication [43]. Paper is a fabric matrix made of cellulose, excellent in wicking liquids. Different from the molding-sealing strategy to fabricate other materials, paper microfluidic device could be fabricated by patterning certain area of the paper hydrophobic, which could be
accomplished simply with a printer; water applied to the paper will be guided through the hydrophilic region, which serves as a channel even if the region is open to air [44]. Besides, the fabric matrix of paper could filter out large particles or act as a ‘solid reservoir’ for reagents applied and let dry. When coupled with colorimetric detection methods, paper-based microfluidic devices realize a platform for inexpensive, power free, portable bioassays. Recently, Whitesides et al. demonstrated measuring markers of liver function using a micropatterned paper device designed for blood from a fingerstick [45,46]. In their method, the paper device filter out the blood cells from the sample and transport the filtrate to several detecting spots on the device; the colorimetric result is captured by a smart phone and sent for telemedicine; the device is finally incinerated for disposal of hazardous waste (Figure 4A).

Besides disposable analytical use, paper-based devices have also shown a promising potential in 3D cell cultures. By stacking multiple sheets containing spots of cell-laden ECM gels, versatile configuration of a 3D culture could be precisely generated; moreover, de-stacking the layers by peeling realized convenient examination of the culture result in a true 3D fashion (Figure 4B) [47].

**Outlook**

Working typically at a scale close to the sizes of biological cells, microfluidics opened up a new form of biological research, enabling the power of conveniently manipulating single cells, precisely controlling their surrounding environment, detecting the signal from individual cells, and operating at high-throughput and automated modes. Further growth could be expected in the following trends. First, the co-culture of multiple types of cells in a pre-designed 3D arrangement, mimicking their distribution in a real tissue, would attract significant interests in the study of cell–cell interaction, signaling and other tissue level topics. Second, cell-encapsulated droplets/beads would provide a powerful and high-efficient platform for high throughput screening of genetic information; a shell made of hydrogel is a key element of this approach.
as a controllable encapsulation/release scheme that restricts its content during batch process, e.g., cell lysis, PCR amplification and bead-based sorting, and releases the content on demand for subsequent analyze. Third, establishment of standardized, universal platform for on-chip cell culture and real-time and long-term monitoring of cell response to drugs would greatly promote the global adoption of microfluidic technology as a standard tool in drug discovery and culture-related processes. Last but not least, the commercialization of portable and disposable microfluidic devices, most likely made of paper or plastics, would greatly benefit public health and security in various forms.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


A general platform for microfluidic studies involving 2D and 3D cell culture, based on a PDMS-hydrogel hybrid device. Biochemical and biophysical stimuli can be precisely applied to multiple cell types interacting over distances of <1 mm, thereby mimicking many aspects of the in vivo microenvironment.


Demonstrated an approach using a valve-controlled 3D laminar flow to tessellate and code planar soft materials that is scalable and continuous. The method does not involve substrate support or moving device components, and is compatible with a range of biopolymers and different cell types. The generated cell-laden film could be stacked or rolled for 3D cell culture.


This review covered the advances in the continuous microfluidic encapsulation of cells in droplets and microgels till 2011.


Demonstrated a simple one-step approach that exploits a versatile host-guest system and uses microfluidic droplets to generate porous microcapsules with easily customizable functionality. The generated capsules are amenable to on-demand encapsulant release. The internal chemical environment can be probed with surface enhanced Raman spectroscopy.


Illustrated a type of test useable for a range of assays in resource-poor settings, in which the entire assay was completed on a paper-based microfluidic device and analyzed with the help of a smart phone.
