Multiphase microfluidic synthesis of micro- and nanostructures for pharmaceutical applications

Rui Ran, Qi Sun, Thejus Baby, David Wibowo, Anton P.J. Middelberg and Chun-Xia Zhao*

*Australian Institute for Bioengineering and Nanotechnology, The University of Queensland,
St Lucia, QLD 4072*

*Corresponding author.

Tel.: +61 7 3346 4263;

Fax: +61 7 3346 4197.

E-mail address: z.chunxia@uq.edu.au (C.-X. Zhao).

Submitted to *Chemical Engineering Science*

Abstract

Multiphase microfluidics has attracted significant interest in making micro- and nanostructures for various applications because of its capabilities in precisely controlling and manipulating a small volume of liquids. In this review, we introduce the recent advances in making micro- and nanostructures for pharmaceutical applications, including microparticles and microcapsules for controlled release, nanoparticles for drug delivery and microgels for 3D cell culture. With the development of more advanced microfluidic systems, the research focus in this field has shifted from making simple micro- and nanostructures to multifunctional systems to achieve more desirable functions. However, these multifunctions may lose their advantages that have been demonstrated in vitro once they are applied in vivo or later in human. The key challenge is a lack of fundamental understanding of the interactions between the micro- and nanomaterials and the biology systems. Consequently, the translation of these advanced materials lags far behind their extensive laboratory research. To better understand the micro- or nano-bio interactions, the development of new in vivo-mimicking models is imperative. Microfluidics has demonstrated its great potential in creating physiologically relevant models including 3D cell culture, tumor-on-a-chip and organs-on-a-chip. Therefore, efforts towards developing 3D cell culture and biomimetic chips including tumor-on-a-chip and organs-on-chips for faster and reliable evaluation of these micro- and nanosystems are also highlighted.

Keywords: Microfluidics; Microparticles; Nanoparticles; Tumor-on-a-chip; Organs-on-a-chip

1. Introduction

There has been a long-standing need for improved pharmaceutical formulations that are capable of delivering therapeutics (drugs, genes, and biomolecules) at a controllable rate to specific sites (cell, tissue or organ). Novel carriers hold great promise in achieving enhanced bioavailability, reduced cytotoxicity, targeted delivery, controlled release, and improved efficacy that can ultimately result in desired therapeutic responses in the body. Among these carriers, micro- (Kurmi et al., 2010; Leong and Wang, 2015; Skorb and Möhwald, 2014) and nano- (Gref et al., 2012; Peer et al., 2007; Schroeder et al., 2010; Wang et al., 2012) structures have attracted significant research interest because of their tunability in physical (size, structures, porosity, and mechanical strength) and chemical (compositions, reactivity, biocompatibility, and biodegradability) properties, and flexibility in integrating different functions, such as for active/passive targeting, stimuli-responsive release, and diagnostics/imaging. To enable their full potential in practical pharmaceutical applications, the development of advanced and robust platform technologies for the manufacture of micro- and nanostructured materials with high degree of uniformity (size, size distribution, and shape), batch-to-batch reproducibility and scale-up possibilities, becomes highly essential.

Microfluidics as modern technology has been transforming some of the industrial practices for controllable synthesis of multicomponent carrier systems with sophisticated structures and multiple functions (Riahi et al., 2015; Vladisavljevic et al., 2013). Multiphase microfluidics involves two or more partially or immiscible fluids in contact. It has been commonly applied to enhance mixing, increase mass transfer across phase boundaries, and reduce dispersion (Günther and Jensen, 2007; Zhao and Middelberg, 2011). Also, microfluidics has unique characteristics such as pico- to nanoliters of reagents, nano- to microseconds of mixing, reaction and self-assembly, real-time monitoring/imaging and direct scale-out. These properties offer significant advantages for relatively low-cost and high-

throughput production of micro- (microparticles, microcapsules, and microgels) (Gañán-Calvo et al., 2013; Shim et al., 2013; Zhao, 2013) and nanocarriers (polymeric nanoparticles, liposomes, and hybrid nanoparticles) (Khan et al., 2013; Khan et al., 2015b; Zhao et al., 2011).

Despite the vast amount of efforts in developing various micro-/nano-carriers, their translation from *in vitro* to animal studies (preclinical) and finally human trials (clinical) has been tremendously expensive with long timelines and quite often fails at the later stage of their development even after entering human clinical trials. Until now, only a few nanosystems have been approved by the U.S. Food and Drug Administration (FDA) for human use, for example, Doxil (a liposomal formulation encapsulating Doxorubicin) (Koynova and Tenchov, 2015) and Abraxane (based on the nanoparticle albumin-bound (nab) technology to deliver Paclitaxel) (Qiang et al., 2009) for cancer treatments. One of the hurdles is the lack of robust *in vitro* or *in vivo* systems that can predict the behavior of drugs and carriers in the human body. *In vivo* animal models have been widely used but cannot accurately predict human responses due to inter-species differences. Also, animal experiments are time-consuming, high cost, and have ethical concerns. Microfluidics offers structures and networks at the micrometer length scale comparable to relevant physiological length scales and can incorporate fluid flows and mechanical forces capable of mimicking the *in vivo* microenvironment. Therefore, it enables the investigation of complex interactions between these micro-/nanosystems and biological systems. Furthermore, integration of tumor spheroids or organoids with microfluidics to build tumor-on-a-chip, organs-on-a-chip, and ultimately human body-on-a-chip creates a platform that cannot be achieved by conventional cell and tissue culture in well plates. Thus, organs-on-a-chip can be useful for predicting preclinical and even clinical performances of new therapeutics and micro-/nano-delivery vehicles at early stages of drug development.

In this review, we will critically review the recent advances in making micro- (microparticles, microcapsules, and microgels) and nanostructures (polymeric, liposomes, and hybrid nanoparticles) in microfluidics for therapeutic delivery and controlled release, with a focus on new structures, new functions, and their pharmaceutical applications. We will also highlight recent development in making microgels for 3D cell culture as well as tumor-on-a-chip and organs-on-a-chip, which provide rapid and reliable screening and evaluation tools for accelerating the development of drug delivery systems.

2. Well-controlled synthesis of micro- and nanostructures and their pharmaceutical applications

A wide variety of micro- and nanostructures have been synthesized using microfluidics (Fig. 1), including microparticles, microcapsules, microgels, and nanoparticles made of various materials including synthetic polymers, natural polymers, lipids, and hybrid materials. This section will introduce the synthesis of these micro- and nanostructures and their pharmaceutical applications.

Fig. 1 Types of micro- and nanostructures synthesized using microfluidics

2.1 Synthesis of microstructures and their applications

2.1.1 Microparticles

Microparticles herein are defined as particles with a size ranging from 1 to 1000 μm . Microfluidics offers several unique advantages for preparing microparticles, such as precise control over particle size, surface morphology, and high flexibility in making microparticles with various materials and different structures. Microfluidic approaches have been widely explored for making a wide variety of microparticles in the past decade. Initially, researchers have been focusing on making microparticles made of different materials for encapsulation of one drug or more for slow release applications, as well as precise control over their size and surface texture (Fig. 2). Then the studies moved on to make microparticles with more complex structures, for example, core-shell microparticles, Janus particles, and even more complex shapes, coupled with triggered release by pH, temperature, light, etc. Recently, the research focus has been shifting from the development of different microfluidic devices and methods for making various microparticles for encapsulation and controlled release (Bokharaei et al., 2016) to the fabrication of microparticles made of new materials, more complex structures, and multiple functions for more sophisticated applications, such as multistage pH-responsive properties, precise control over drug loading into each domain, versatile loading of different drugs and tailored release kinetics. Several articles reviewed the synthesis of polymeric microparticles (Wang et al., 2014a), non-spherical polymeric microparticles (Baah et al., 2012), microparticles based on emulsion templates (Wong et al., 2006; Zhao, 2013), porous microspheres (Duncanson et al., 2012b; Fan et al., 2013), designer microparticles for encapsulation and controlled release applications (Duncanson et al., 2012a; Kong et al., 2012). In this review, we highlight the new development of this field since 2013.

Fig. 2 Development of microparticles for various applications.

Microparticles with complex shapes

Microparticles are generally fabricated by a strategy called droplet-to-particles through different solidification methods, including UV polymerization (Khan et al., 2015a; Liu et al., 2016a; Xue et al., 2015), solvent evaporation (Min et al., 2016), spray drying (Liu et al., 2015a; Liu et al., 2016c; Liu et al., 2012) and ionic cross-linking (Chan et al., 2009; Liu et al., 2016b). In addition to these conventional approaches, which have been widely used to fabricate spherical particles, a number of other ways to make non-spherical polymer microparticles have been developed. Microparticles with complex 3D shapes can be made through the simple tuning of mold swelling and capillarity using 2D micromolds (Choi et al., 2015b). A sequential micromolding method was developed to make multicompartmental polymeric microparticles. Basically, primary and secondary compartments were formed in micromolds sequentially based on surface-tension induced droplet formation followed by simple photopolymerization (Choi et al., 2015a). Additionally, 3D-printing (Chen et al., 2014a) and stop-flow lithography (An et al., 2013) methods have also been applied to make complex multicompartmentalized microparticles.

Janus particles and multicompartamental particles

Instead of relying on the physical confinement to generate complex microparticles, a number of new strategies have also been developed. Drug-loaded Janus particles can be prepared using side-by-side microcapillary devices (Khan et al., 2014). Min et al. made multiple compartment microparticles through phase separation (Min et al., 2016). Uniform solvent droplets containing two immiscible polymers (one is biodegradable, and the other is pH-responsive) were generated in a capillary microfluidic device. With the evaporation of the solvent the two polymers underwent phase separation followed by co-solidification, leading to the formation of microparticles with distinct compartments. When two drugs with different hydrophobicity were initially dissolved in the solvent along with the two polymers, they were able to concentrate in the compartment that they had high affinity. Because of the properties of the two polymers, they can provide unique release profiles of the model drugs. Hayakawa et al. also reported the generation of multicompartamental microparticles with complex shapes (Hayakawa et al., 2016). Their method was based on the Marangoni and diffusional flows of droplets containing two or more compositions through double-, triple- or even septuple-glass capillary-based centrifugal microfluidic device. Ekanem et al. produced biodegradable poly(DL-lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) microparticles with different shapes, internal structures and surface morphologies (Ekanem et al., 2015). Janus and hemispherical particles were generated by solvent-induced phase separation, and less-porous particles and porous golf-ball-like particles were made by adding non-clay or porogen in the dispersed phase. Dong et al. made hybrid organic-inorganic (PLGA-TiO₂) microparticles with tunable surface textures by changing the mass ratio between titanium tetrakisopropoxide (TTIP) and PLGA in the droplet phase. The hydrolysis of TTIP formed a thin layer of TiO₂ and then a wrinkled surface formed as a result of the removal of organic solvent through extraction (Dong et al., 2015).

Core-shell microparticles

Microparticles with core-shell structures can provide controlled release profiles, such as more sustained release, stimuli-responsive release. Monodisperse PLGA-alginate core-shell microspheres were fabricated using double emulsions as the templates in a microcapillary device (Wu et al., 2013). The alginate-shell was used to modulate the release profile. Uniform gelatin-alginate core-shell microparticles were designed as a pH-responsive drug carrier. They were synthesized using sodium alginate solution containing gelatin capsules as the dispersed phase (Huang et al., 2014). The alginate-shell was then solidified through cross-linking in a CaCl_2 solution. As the alginate-shell is stable in acid environments, the core-shell microparticles remained intact in gastric solution for more than 3 h. In contrast, the alginate-shell could swell under alkali conditions and then release the drug. This core-shell microparticles are potential for oral drug delivery to the intestine. Thermo-responsive core-shell microparticles were prepared using poly(*N*-isopropylacrylamide) (PNIPAM) as the core and porous ethyl cellulose shell embedded with PNIPAM gates (Yu et al., 2012). In addition to triggers like pH, temperature, light, etc., external fields can also be used as a trigger. For example, perfluorocarbon-alginate core-shell microparticles remained intact up to 21 days but were disrupted easily after exposure to ultrasound for 15 mins (Duarte et al., 2014).

Multifunctional microparticles

Multifunctional microparticles have also attracted significant interests in the past several years. Multidrug-loaded microparticles demonstrated a sustained release of both drugs (Leon et al., 2015). Quantum dots (QD)-encoded poly(ethylene glycol) diacrylate (PEGDA) microbeads with the size ranging from 7 to 120 μm were synthesized for suspension assay (Liu et al., 2016a). Magnetic-fluorescent Janus alginate microparticles were made to achieve the luminescent labeling and magnetic separation. The particles were made

using two alginate streams with one doped with Fe_3O_4 nanoparticles and the other one with CdSe/ZnS quantum dots as the dispersed phase (Chan et al., 2009). Kim et al. developed multifunctional alginate microspheres for magnetic resonance imaging (MRI) and a sustained drug-release profile by encapsulating magnetic clusters of ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles and a drug (amonafile) (Kim et al., 2015a). Zhang et al. developed multifunctional nano-in-micro particles for orally administered targeted drug delivery (Zhang et al., 2014). Porous silicon nanoparticles (PSi) loaded with a drug was modified using a mucoadhesive polymer, poly(methyl vinyl ether-*co*-maleic acid) (PMVEMA) through a poly(ethyleneimine) (PEI) as a linker. The modified PSi-PEI-PMVEMA nanoparticles were then encapsulated inside a pH-responsive hydroxypropyl-methylcellulose acetate succinate based polymer (ASHF). The nano-in-micro systems were further engineered for multistage pH-responsive controlled multidrug delivery. A drug atorvastatin was firstly loaded into the PSi which were further encapsulated into a pH responsive polymer microparticles containing another drug celecoxib. Therefore, this nano-in-micro drug delivery system remained stable in acidic somatic conditions thus prevent any premature release in the gastrointestinal (GI) tract, while the alkaline pH condition in the upper intestinal region triggered the drug release. Instead of using a multistep process by first forming nanoparticles *ex situ* followed by producing nano-in-micro particles in microfluidic devices, a semi-continuous process was developed for making nano-in-micro Trojan particles combining an elongational-flow micromixer for producing polymerizable nanoemulsions coupled with a co-axial microcapillary for forming microparticles (Khan et al., 2015a).

2.1.2 Microcapsules

Microcapsules refer to core-shell microstructures composed of a solid shell that surrounds a core-forming space available to entrap active molecules. The core-shell structure of microcapsules offers several advantages over their solid and porous counterparts. The core

allows the encapsulation of various cargoes, ranging from drugs (Li et al., 2015; Liu et al., 2014; Vasiliauskas et al., 2015; Yang et al., 2009; Yang et al., 2014), proteins (Pessi et al., 2014; Yeh et al., 2011; Zhang et al., 2013), vitamins (Liu et al., 2013; Yeh et al., 2013), cells (Ferreira et al., 2013; Kim and Kang, 2014; Mendes et al., 2012) to imaging agents (Abbaspourrad et al., 2013a; Abbaspourrad et al., 2013b; Gokmen et al., 2009) and magnetic nanoparticles (Ge et al., 2014; Liao and Su, 2010; Yang et al., 2009). Also, microcapsules with gas filled in the core have attracted interests (Abbaspourrad et al., 2013c; Yoon et al., 2015) especially for acoustic imaging as they are more echogenic than liquid-filled microcapsules. The shells of microcapsules, on the other hand, function as protective barriers for the encapsulated cargo, and its compositions and synthesis conditions determine the physical (e.g., mechanical strength, shell thickness, and diffusivity) and chemical (e.g., biocompatibility, biodegradability, and functionality) properties of the resultant microcapsules. Droplet-based microfluidics has been widely used to fabricate microcapsules. Typically, single or double emulsions are used as templates which are then solidified to form microcapsules through a number of solidification methods including: evaporation-induced solidification (Abbaspourrad et al., 2013a; Abbaspourrad et al., 2013b), extraction-induced solidification (Foster et al., 2010; Liao and Su, 2010; Vasiliauskas et al., 2015), UV polymerization (Abbaspourrad et al., 2013c; Liu et al., 2014; Zhang et al., 2013; Zieringer et al., 2015), and layer-by-layer deposition (Gokmen et al., 2009).

Microcapsules capable of releasing encapsulated cargoes in a controlled and predictable manner are essential for pharmaceutical applications. Therefore, stimuli-responsive materials are often incorporated into the core or the shell. The release of cargo can be triggered by external stimuli, such as, acids/bases (Abbaspourrad et al., 2013b; Liu et al., 2013; Vasiliauskas et al., 2015; Yang et al., 2014), organic solvents (Abbaspourrad et al., 2013a), biomolecules (Zhang et al., 2013), and magnetic fields (Ge et al., 2014; Liao and Su, 2010;

Yang et al., 2009). For example, Zhang et al. developed thermo- and glucose-responsive microcapsules based on double emulsion templated methods (Zhang et al., 2013). The insulin encapsulated microcapsules exhibited reversible and repeated swelling/shrinking response to glucose concentration at 37°C hence triggering insulin release. Although microcapsules with triggered-release properties have been developed, cargo leakage still presents a technological challenge which may compromise their applications. To minimize unwanted cargo loss, Zieringer et al. utilized perfluoropolyether as the shell material, which has been demonstrated to retain 98% of the encapsulated small molecules (i.e., CaCl_2) over four weeks and the salts can be released using osmotic stress-induced bursting mechanism (Zieringer et al., 2015). Moreover, the porosity and functionality of microcapsules can be engineered by incorporating silica particles on the shell during the synthesis of microcapsules, and the porosity can then be formed after removal of the silica particles using alkaline solutions. To enhance the utilities of microcapsules in pharmaceutical applications, site-specific targeted delivery of microcapsules with direction-specific controlled release of cargo can be used (Ge et al., 2014; Yang et al., 2009). For example, Yang et al. synthesized smart microcapsules of poly(caprolactone) for combined magnetic targeting, fluorescent imaging, and controlled drug release properties by encapsulating Fe_3O_4 nanoparticles, CdTe quantum dots, and anticancer drug tamoxifen, respectively (Yang et al., 2009).

2.1.3 Microgels

Microgels are micrometer-sized gel particles consisting of three-dimensional (3D) polymer networks. Various types of natural and synthetic polymers have been used for making microgels, e.g., alginate (Utech et al., 2015), agarose (Shi et al., 2013), chitosan/dextran (Oh et al., 2014), gel-forming peptides (Tsuda et al., 2010), poly(*N*-isopropyl acrylamide) (PNIPAM) (Seiffert and Weitz, 2010), and poly(2-methacryloyloxyethyl phosphorylcholine) (Park et al., 2014). The polymer-chain chemistry

of microgels determines their hydrophilicity/hydrophobicity which, in turn, determines the types of solutions where the microgels can be dispersed (e.g., in aqueous or organic solvents) as well as the types of cargoes that can be loaded. Due to their open network structure, microgels can sequester functional cargoes to their interstitial spaces by equilibrium partitioning between the solution and microgels phases (Chen et al., 2010). Electrostatic interaction (Chen et al., 2010; Kim et al., 2007), hydrophobic interaction (Chen et al., 2010), hydrogen bonding (Fang et al., 2010), or covalent conjugation (Utech et al., 2015) may play an important role for the adsorption of cargoes into the microgels' polymer-network. The cargoes can be released from the microgels either by passive- or active-diffusion driven by swelling/shrinking of the microgels due to external stimuli such as temperature (Jagadeesan et al., 2011), pH (Lu et al., 2015), and light (Luo et al., 2014). The abilities of microgels to encapsulate and release drugs have enabled them for controlled drug delivery (Hu et al., 2012; Lu et al., 2015). Other functional cargoes, e.g., thermo-responsive nanoparticles (Luo et al., 2014), dyes (Park et al., 2014; Xu et al., 2015), quantum dots and magnetic nanoparticles (Kim et al., 2007), have been encapsulated in microgels, demonstrating their versatile utilities for pharmaceutical applications. Furthermore, microgels have been used for cell encapsulation and 3D cell culture which will be discussed in detail in *Section 3.1*.

Microgels are commonly fabricated using droplet-based microfluidics. Droplets containing a pre-microgel mixture are formed in an immiscible carrier phase, and then turn in microgel particles through various subsequent droplet gelation methods, such as chemically (e.g., by photo-polymerization (Jagadeesan et al., 2011; Lu et al., 2015; Park et al., 2014) and free-radical copolymerization (Seiffert, 2012)) or physically (e.g., by temperature changes (Shi et al., 2013) and ionic cross-linking (Guo et al., 2011; Miyama et al., 2013)). The size of microgels depends on the size of the emulsified droplets, thus is tunable by adjusting the flow

rates of the fluids during the microfluidic droplet formation or by controlling the dimensions of the microfluidic devices. In addition to particle size, well-defined mesh size and its homogeneity within microgels play important roles in the precision regulation of the release kinetics in pharmaceutical applications. The mesh size of microgels is characterized by the molecular weight between cross-links (Park et al., 2014). Moreover, the shape of the droplets generated by microfluidic devices is generally spherical because of the interfacial tension between the continuous and dispersed phases. By controlling interfacial tension and viscoelastic properties of the droplets, microfluidic droplet templating can also serve to form microgels with complex morphology, including yarn-ball (Miyama et al., 2013), pear-like, mushroom-like, red blood cell-like (Hu et al., 2012), ellipse, triangle, and dumb-bell (Seiffert, 2012). These non-spherical microgels revealed unique properties such as anisotropic responses to an external force, large surface area.

2.2 Synthesis of nanostructures and their applications

2.2.1 Synthetic polymer nanoparticles

Over the years, a variety of synthetic polymers has been explored for the preparation of nanoparticles (NPs) as drug delivery nanocarriers using microfluidic approaches. Poly(lactic-co-glycolic acid) (PLGA) (Hines and Kaplan, 2013; Makadia and Siegel, 2011), poly(lactic acid) (Kolishetti et al., 2010), poly(methyl methacrylate) (PMMA) (Anton et al., 2012) and Pluronic F-127 (Capretto et al., 2011) have gained paramount attention owing to their biocompatibility and biodegradability. PLGA is one of the most popular and extensively researched synthetic polymer for pharmaceutical applications and is considered as the “gold standard” for controlled release studies. PLGA-based NPs have a broad range of applications because of their unique properties such as versatile degradation kinetics, non-toxicity, biocompatibility, and the U.S. Food and Drug Administration (FDA) approval for human use. Compared to traditional beaker methods, microfluidic technology offers a reproducible and

controllable platform for rapid NP synthesis with wide-ranging properties (Karnik et al., 2008; Zhao et al., 2011). The ability of microfluidic systems to rapidly mix reagents providing homogeneous reaction environments and to vary the reaction conditions continuously allowing reagent addition during the progress of a reaction are the key features that make them attractive for the NP synthesis.

Two kinds of microfluidic methods have been developed for the synthesis of PLGA NPs i.e. droplets and flow focusing. Hung et al. employed a droplet-based approach to precipitate the PLGA NPs. Water and PLGA solution (in an organic solvent, dimethyl sulfoxide (DMSO)) were sheared by mineral oil into monodispersed droplets, then fused in a widening chamber to initiate the mixing hence PLGA NPs precipitation due to the solubility difference (PLGA solubility in water is lower than in DMSO) (Hung et al., 2010). PLGA NPs of up to three orders of magnitude difference in size (70 to 482 nm) can be synthesized by changing the flow rate ratio of water to the PLGA–DMSO (1:1 to 1:4).

Flow-focusing microfluidics is another widely-used approach to produce PLGA NPs. Solvents such as acetonitrile with PLGA dissolved is hydrodynamically focused by a non-solvent (water or buffer) to achieve tunable and rapid mixing, leading to nanoprecipitation of NPs. Sun et al. developed a microfluidic origami chip with different geometries to produce monodispersed doxorubicin (Dox)-loaded PLGA NPs with tunable size (70–230 nm) in a single nanoprecipitation step through the rapid mixing of PLGA–Dox with water (Fig. 3A) (Sun et al., 2013). The chip was fabricated by bonding two poly(dimethylsiloxane) (PDMS) layers after oxygen plasma that could be folded manually to achieve different geometries such as an arc and double spiral. The design achieved a throughput of 1200 mg of NPs per day at a maximum flow rate of 2.5 mL/h for 2% PLGA–Dox solution. Karnik et al. prepared docetaxel (Dtxl)-loaded poly(lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol) (PLGA–PEG) NPs through rapid and tunable 2D hydrodynamic flow focusing (HFF) in microfluidic

channels (Karnik et al., 2008). By varying the flow rates, polymer composition and polymer concentration, the Dtxl-loaded PLGA-PEG NPs had a smaller size, a lower dispersity ($\mathfrak{D}$), and a higher drug loading with a slower release than the ones synthesized using bulk preparation methods. However, one of the challenges of using 2D HFF was that the NPs had the tendency to aggregate, thus clogging the channel, when the PLGA block has a molecular weight (M_w) higher than 45 kDa, resulting in an irreversible failure. Rhee et al. designed a 3D HFF device composed of a monolithic single layer with three sequential inlets for vertical focusing in combination with a cross junction for horizontal focusing (Fig. 3B) (Rhee et al., 2011). NPs of different polymer concentrations and M_w that were otherwise difficult to assemble by either 2D HFF or bulk preparation methods were successfully fabricated using this 3D HFF method. Mathematical modeling along with simulation and confocal microscopy led to the prediction of optimal ranges for the reproducible synthesis of PLGA NPs, so realized the prevention of fouling and enhancing the robustness of operation. Valencia et al. developed a modified 3D HFF for the on-chip combinatorial synthesis of targeted polymeric NPs, thereby combining the 3D HFF and micromixing (Fig. 3C) (Valencia et al., 2013). PLGA-PEG NPs of various size (25 to 200 nm), surface charge (-20 to $+20$ mV), target ligand density (0 to $\sim 10^5$ ligands/ μm^2), and with an anticancer-drug loaded (0 to 5%) were synthesized on-chip in a rapid and reproducible manner. A library of 45 different formulations was then screened *in vitro* and *in vivo*. Lim et al. developed a microfluidic parallelization method using a multilayer 3D HFF to enable high-throughput NP synthesis with tunable particle size (Fig. 3D) (Lim et al., 2014). The production rates can be increased considerably by using eight 3D HFFs (84 mg/h) in parallel in comparison with a single 3D HFF (4.5 mg/h) at similar flow conditions. Furthermore, low M_w PLGA block (10 kDa) yielded small NPs (~ 13 nm), while high M_w PLGA block (95 kDa) and high polymer concentration produced large NPs (~ 150 nm), with both having uniform size distribution and

minimum batch-to-batch variability. This is appealing as smaller NPs are able to penetrate *in vivo* into solid tumors more effectively while acknowledging the fact that it is highly challenging to fabricate drug-loaded polymeric NPs with controlled-release properties in that size range via bulk synthesis routes.

Fig. 3 Designs of microfluidic devices for the synthesis of synthetic polymer nanoparticles.

(A) Microfluidic origami chip: (a) Schematics of a microfluidic origami chip for the synthesis of monodisperse doxorubicin (Dox)-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs); (b) An arc geometry obtained by manually folding the origami chip; (c) A double spiral geometry obtained from the same chip. Reprinted from (Sun et al., 2013), Copyright (2013), with permission from Elsevier. (B) Schematics of a 3D hydrodynamic flow focusing (HFF) device consisting of three sequential inlets for vertical focusing and a separate inlet for side sheath flows. Reproduced with permission from (Rhee et al., 2011). Copyright (2011) John Wiley & Sons Inc. (C) Microfluidic platform for the rapid synthesis of PLGA-PEG NPs. Reprinted with permission from (Valencia et al., 2013). Copyright (2013)

American Chemical Society. (D) 3D HFF microfluidic device for the parallel synthesis of PLGA-PEG NPs in acetonitrile (ACN). Reprinted from (Lim et al., 2014), Copyright (2014), with permission from Elsevier.

2.2.2 Natural polymer nanoparticles

Natural polymers have been widely exploited for pharmaceutical applications owing to their marvellous properties. There are mainly two types of frequently used natural polymers for nanoparticle preparation: chitosan and alginate.

Chitosan is a hydrophilic polysaccharide derived from the exoskeleton of insects, crustaceans, and fungi. It has great potential in various applications due to its gel-forming capability, high adsorption capacity, biocompatibility, non-toxicity, low-immunogenicity, and biodegradability (Dash et al., 2011; Park et al., 2010). The reactive functional groups (primary amine and hydroxyl groups) of chitosan allow specific chemical modifications to form different derivatives for drug delivery applications (Prabaharan, 2015). Microfluidics is attractive for making chitosan nanoparticles. Although fast mixing in microfluidics remains a challenge due to its laminar flow characteristics (Rhee et al., 2011), it has been demonstrated computationally (Kamat et al., 2015) and experimentally (Cetin et al., 2014; Chen et al., 2014b; Majedi et al., 2014) that introducing active mixers can facilitate rapid mixing through chaotic advection and diffusion. Cetin et al. designed a microfluidic device (Fig. 4A) with micro-obstacle structures to enhance mixing thus enabling high throughput production (Cetin et al., 2014). Majedi et al. made a T-shaped microfluidic device with a hydrodynamically focused flow that had well-controlled mixing regime to synthesize monodispersed chitosan NPs (Fig. 4B) (Majedi et al., 2014). The physical (e.g., size and zeta potential) and chemical (e.g., biocompatibility) properties of the chitosan NPs can be tuned by controlling the mixing

time and by using controlled pH-changes in water to drive the assembly of NPs, respectively. Hydrophobic anticancer drugs, like paclitaxel, can be encapsulated at high efficiency (>95%), and the drug's potency can be preserved much better than conventional encapsulation methods. These nanoparticles synthesized in microfluidic devices had lower release rate at pH 7.4, which was desirable for long-term circulation stability owing to their compact nanostructures, and higher release rate at pH 5.5 at tumor sites. Additionally, chitosan NPs have also been used as an adjuvant. For example, chitosan/cytosine-phosphodiester-guanine oligodeoxynucleotide (CpG ODN) NPs prepared using a microfluidic method exhibited enhanced cellular uptake and immunostimulatory response in comparison to the NPs synthesized via the conventional bulk mixing method (Chen et al., 2014b).

Fig. 4 Microfluidic devices for the synthesis of chitosan nanoparticles. (A) Microfluidic channel network with five inlet reservoirs. Copyright 2013 by ASME. Reproduced with permission from (Cetin et al., 2014). (B) Schematic representation of a T-shaped microfluidic device which is used to hydrodynamically flow focus high molecular weight chitosan supplement (HMCS) using a sheath flow of water at basic pH (top). Transmission electron

micrograph (TEM) images of nanoparticles prepared from HMCS with three different degree of substitution (a–c). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission from (Majedi et al., 2014).

Alginate is a water-soluble linear anionic polysaccharide extracted from the brown seaweeds. It has potentials for numerous pharmaceutical and biomedical applications due to its attractive properties such as mucoadhesivity, biodegradability, and biocompatibility. Alginate NPs can be formed by polyelectrolyte complexation in which calcium (Ca)-alginate pre-gels interact with cationic polymers thus forming NPs through ionic interactions (Paques et al., 2014). However, conventional non-homogeneous mixing methods result in micro aggregates of broad size distribution that prevent their use in practical applications. To minimize aggregation and narrow size distribution, Kim et al. fabricated alginate NPs using the polyelectrolyte complexation between anionic Ca-alginate pre-gel and cationic poly-L-lysine (PLL) in a microfluidic mixing device (Fig. 5A) (Kim et al., 2015c). By changing the flow rates of the Ca-alginate pre-gel and PLL solutions, the size of alginate NPs could be controlled (380 to 520 nm). Another method to form alginate NPs was through a microbubble bursting process in a T-junction microfluidic device (Fig. 5B) (Elsayed et al., 2015). The alginate NPs can be produced with different sizes (80 to 200 nm) depending on the viscosity of the solution used to prepare the alginate microbubbles.

Fig. 5 Microfluidic devices for the synthesis of alginate nanoparticles. (A) T-junction microchannel for creating alginate microbubbles (i) and schematic of the alginate microbubble bursting to form alginate nanoparticles as shown in the scanning electron micrograph (SEM) image (scale bar: 1 μm). Reprinted from (Elsayed et al., 2015), Copyright (2014), with permission from Elsevier. (B) Microfluidics-aided polyelectrolyte complexation. The transmission electron micrograph (TEM) image shows alginate nanoparticles (scale bar: 1 μm). Reprinted from (Kim et al., 2015c), Copyright (2015), with permission from Elsevier.

2.2.3 Liposomes

Liposomes are spherical-like vesicles with an aqueous core entrapped by one or more lipid bilayers. Since synthesized in the 1960s for the first time, liposomes have been

intensively studied as hollow and flexible nanocarriers because of their superior biocompatibility and biodegradability. In the past decades, bulk synthesis methods, such as freeze-thaw cycling, film hydration, alcohol injection and detergent depletion, have been developed (reviewed in (van Swaay and deMello, 2013)). However, these conventional methods usually require post-processing steps, such as sonication or extrusion, to homogenize the size as well as to narrow the size distribution, which may cause batch-to-batch variability thus limit their commercial applications. Microfluidic technology has led to a new way of redefining the “exquisite and dynamic control” of the preparation of liposomes. A number of microfluidic methods became available, including pulsed jetting (Funakoshi et al., 2007), droplet-based microfluidics (Kong et al., 2015; Ryan et al., 2012), microfluidic hydrodynamic focusing (MHF) (Jahn et al., 2004) and vertical flow focusing (VFF) (Hood and DeVoe, 2015). The pulsed jetting and droplet-based microfluidics are mainly designed for producing micrometer-sized lipid vesicles, and the MHF and VFF methods are widely used for making nanoscale liposomes. In the MHF method, the central stream of the lipids solutions meets with the two side channels containing an aqueous buffer and then initiates the self-assembly of the liposomes (Fig. 6) (Jahn et al., 2004). Because of the fast mixing in the MHF, liposomes with high reproducibility and controllable size were fabricated (Belliveau et al., 2012; Hood et al., 2014; Jahn et al., 2004). Higher flow rate ratio of the volumetric flow of the aqueous buffer to that of the lipids solution results in smaller size and better monodispersity (Jahn et al., 2013; Jahn et al., 2010; Mijajlovic et al., 2013; Phapal and Sunthar, 2013). Furthermore, a VFF approach using a large microchannel aspect ratio (channel depth to channel width), up to 100:1, was developed for producing liposomes at a throughput of nearly 100 mg/h (Hood and DeVoe, 2015), which makes the microfluidic technology promising for fabricating liposomes for practical applications.

Fig. 6 Schematic of the synthesis of liposome in microchannel by using 2D (A) and 3D (B) microfluidic hydrodynamic focusing (MHF) methods. Dissolution of alcohol into water leads to a decreased solubility of the lipid materials. As a consequence, the lipid gradually aggregates into bilayer planar disc and eventually close into liposomes. Reprinted with permission from (Jahn et al., 2004). Copyright (2004) American Chemical Society.

Liposomes have been developed as nanocarriers by loading various functional cargoes for pharmaceutical applications, including drugs (Hood et al., 2014; Kastner et al., 2015) and genes (Balbino et al., 2013; Belliveau et al., 2012; Chen et al., 2012). The cargo can be loaded into liposomes either by active or passive loading. Hood et al. prepared doxorubicin (Dox)-loaded liposomes in an integrated microfluidic mixing device using a one-step continuous-flow process which combined liposome synthesis and drug loading (Hood et al., 2014). Briefly, liposomes were first formed in an MHF section and then flowed to a counter-flow microdialysis element where buffer exchange occurred. The resulting pH shifting of the solution enabled steep transmembrane ion gradients prior to remote drug-loading in the incubation zone (active loading) (Fig. 7). This process produced ‘coffee bean’-like liposomes having a diameter of 190 nm and Dox-encapsulation efficiency of 72%. Kastner et al. loaded a hydrophobic drug, propofol, into liposomes by simply mixing the hydrophobic drug and

lipid solution before injected them to the microfluidic device (passive loading). This method generated drug-loaded liposomes with a tunable size (50–450 nm) and higher loading efficiency (41% mol) compared to liposomes formed using the conventional sonication method (Kastner et al., 2015). Their drug release profile showed an initial release of 40% of the encapsulated drug in 1 h, followed by a sustained release over another 16 h-period. Other than chemotherapy drugs, genes can be loaded into liposomes for *in vivo* delivery. Chen et al. encapsulated siRNA into cationic liposomes using a microfluidic device with a special geometry design, i.e., staggered herring-bone micromixer (Chen et al., 2012). The resulting liposomal siRNA had a diameter of 60 to 90 nm with a narrow size distribution and high encapsulation efficiency (approximately 80%). The *in vivo* mice experiments using the liposomal siRNA showed more than 90% target gene silencing efficiency. Additionally, liposomes can be functionalized, e.g., with PEG and folate (Hood et al., 2013; Ran et al., 2016), using similar microfluidic approaches to enhance their performance for targeted delivery.

Fig. 7 Schematic of the microfluidic synthesis of nanoscale liposome in-line with buffer exchange via microdialysis and loading of doxorubicin drug. Vertical (A) and lateral (B) views of the microfluidic device. Reproduced from (Hood et al., 2014) with permission from The Royal Society of Chemistry.

2.2.4 Hybrid nanoparticles

Hybrid nanoparticles possess core-shell structures which can be classified as inorganic-core organic-shell (Herranz-Blanco et al., 2015), organic-core inorganic-shell (Chen et al., 2010) and organic-core organic-shell (Fang et al., 2010) NPs. Among them, organic-core organic-shell, lipid-polymeric nanoparticles (LPNs) have received considerable attention for pharmaceutical applications. LPNs are composed of a polymer core and a lipid shell combining the advantages of both polymeric nanoparticles (e.g., sustained release and high drug encapsulation efficiency) and lipid vesicles (e.g., excellent stability and biocompatibility) (Zhang et al., 2008). Traditionally, the synthesis of LPNs involves two

steps, that is, separately preparing polymeric nanoparticles and aqueous lipid films/liposomes, followed by constructing core-shell nanoparticles through extrusion, sonication, or electrostatic interaction (reviewed in (Hadinoto et al., 2013)). In contrast with the bulk synthesis methods, microfluidic approaches combine nanoprecipitation of polymeric materials and self-assembly of the lipid shell in a single step. So they offer advantages such as minimal preparative steps, uniform size, better control over the hybrid particles' physicochemical properties (e.g., size, zeta potential, and shell stiffness), adjustable throughput and superior reproducibility (Table 1). For example, Valencia et al. synthesized stable and homogenous LPNs having tunable size (35–180 nm) and zeta potential (–10 to +20 mV) (Fig. 8) (Valencia et al., 2010). More importantly, their shell rigidity was controlled to regulate the cellular uptake. Sun et al. prepared LPNs having structures of PLGA–lipid and PLGA–water–lipid (by altering the injection order of the PLGA and lipid–PEG organic solutions in the microfluidic chips) which had Young's modulus of 1.2 and 0.76 GPa, respectively (Sun et al., 2015). They found that the more rigid NPs had a much higher cellular uptake than the less rigid ones. To achieve the high-throughput synthesis of LPNs, a microfluidic device was designed to operate at high Reynold numbers (up to 150) using controlled microvortices. It can achieve a production rate of 3 g/h, which was 1000 times higher than the diffusion mixing in a microfluidic flow-focusing pattern and 200 times faster than convective mixing in a Tesla-type mixer (Kim et al., 2012).

Fig. 8 Schematic of the synthesis of lipid-polymeric nanoparticles composed of poly(lactic-co-glycolic acid) (PLGA)-core lipid-shell (in *blue arrow*) and quantum dots-core lipid-shell (in *red arrow*) hybrid nanoparticles in the microchannels and the corresponding electron microscopy images. Adapted with permission from (Valencia et al., 2010). Copyright (2010) American Chemical Society.

Table 1 Representative examples of lipid-polymeric hybrid nanoparticles fabricated using microfluidic, and their associated components and physical properties.

Polymer core	Lipid shell	Size (nm)	Zeta potential (mV)	Throughput [†]	Ref.
NIPA	DPPC, cholesterol, DCP	180–280	n/a	Low	(Hong et al., 2010)
PLGA	Lecithin, DSPE-PEG	35–180	–20 to +10	Low	(Valencia et al., 2010)
PLGA	DPPC, DSPE-PEG, cholesterol	103–106	n/a	Low	(Zhang et al., 2015)
PLGA	Lecithin, DSPE-PEG	30–170	n/a	High	(Kim et al., 2012)
PLGA	EPC, DSPE-PEG	80	–40	High	(Fang et al., 2012)
PLGA	DPPC, cholesterol, DSPE-PEG	40	0	Low	(Sun et al., 2015)

[†]High throughput: ≥ 1 g/h; low throughput: < 1 g/h. Abbreviation: PLGA, poly(lactic-co-glycolic acid); DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy(polyethylene glycol); DPPC, dipalmitoyl-*sn*-glycero-3-phosphocholine; NIPA, *N*-isopropylacrylamide; DCP, dihexadecyl phosphate; EPC, L- α -phosphatidylcholine (egg PC); n/a, not available.

LPNs show promising prospect in the delivery of both hydrophilic and hydrophobic drugs such as doxorubicin (Wong et al., 2006; Zhang et al., 2015) and docetaxel (Liu et al., 2010), respectively. Drugs can be easily loaded by mixing with the polymer or the lipids in the microfluidic device. Zhang et al. encapsulated doxorubicin and combretastatin A4 (CA4) into PLGA-based LPNs for cancer treatment, and demonstrated the effectiveness of this drug carrier in inducing significant cell apoptosis and tumor weight loss. Moreover, they found that PLGA nanoparticles covered with lipid-monolayer-shells, rather than lipid-bilayer-shells, demonstrated better anti-tumor efficacy (Zhang et al., 2015). Mieszawska et al. co-loaded doxorubicin and sorafenib into PLGA-based LPNs using a microfluidic approach with loading efficiencies of 25.6% and 66%, respectively (Mieszawska et al., 2013). The resultant drug-loaded LPNs had a diameter of approximately 85 nm with low polydispersity (~ 0.1). *In vitro* drug release profile showed a significant sustained release of both doxorubicin and sorafenib within a period of 21 days. *In vitro* cytotoxicity study demonstrated that this dual drug-loaded LPNs led to 72% inhibition against human colon cancer cell line (LS174T), and *in vivo* images demonstrated a high accumulation of the LPNs at the tumor site. In addition to loading drugs, nanoparticles such as quantum dots have been loaded into LPNs for imaging (Fig. 8) (Valencia et al., 2010), demonstrating the versatility of hybrid nanoparticles in encapsulating various types of cargoes.

3. Droplet-based 3D microgels for drug screening

A plethora of drug delivery carriers has been rationally designed and developed as described above, which has the potential to enable the use of new drugs as well as to improve

the performance of existing drugs. Their physicochemical properties affect their interactions and behavior within the biological systems (toxicity and efficacy), thus requiring thorough and systematic studies *in vitro* and *in vivo* before their clinical applications. Specifically, the studies include the stability of the carriers within the body, their binding (localization) and internalization at the cellular level, their fate inside the body and their toxicological effects, and their efficacy compared to free drugs (Stirland et al., 2013). Animal models have been used as the gold standard for validating drugs and drug delivery carriers in preclinical testing. However, animal studies are time-consuming, expensive, low-throughput and raising ethical concerns, which limit the pace of clinical translation of new drugs and drug delivery carriers. In contrast, 2D monolayer cell culture models have been commonly used as the *in vitro* models, thereby growing cells (either freshly isolated from human/animal tissues or from immortalized cell lines) on top of a flat substrate. However, they lack the complex 3D *in vivo* microenvironment wherein cells and extracellular matrix exist in well-organized architecture, as well as other issues such as scalability, batch-to-batch variability, and poor predictability. To address these problems, microfluidic technologies offer alternative strategies to develop robust 3D *in vitro* models that better recapitulate what naturally occurs *in vivo*.

3.1 Microgels for cell encapsulation and 3D cell culture

Microgels have been used to develop 3D *in vitro* cell cultures that mimic specific *in vivo* cellular microenvironment by encapsulating living cells and subsequently culturing cells within the gel matrix (attachment, spreading, growth, and proliferation). Various cells have been encapsulated within microgels, such as MCF-7 breast cancer cells (Yu et al., 2015), mesenchymal stem cells (Utech et al., 2015), human umbilical vein endothelial cells (Jung et al., 2014), human epithelial carcinoma cells (Miyama et al., 2013), adenoid cystic carcinoma cells (Shi et al., 2013), and Madin Darby canine kidney cells (Eydelnant et al., 2014). 3D cell cultures in microgels offer several advantages, including tunable shear forces imposed on

cells, easy visualization (e.g., by conjugating fluorescent agent to the microgel's material (Utech et al., 2015)), easy control over the transport of oxygen, nutrients, growth factors and waste (McGuigan and Sefton, 2007) as well as the mechanical and chemical stability in aqueous media such as buffer or cell culture media.

Microgels can be made of synthetic and natural polymers as previously described (Section 2.1.3). Synthetic polymer-based microgels did not gain popularity in cell culture applications due to the harsh conditions involved in the process of microgel preparation, such as strong shear forces, ultraviolet irradiation, and large temperature gradients, which cause severe cell damage or even mortality (Velasco et al., 2012). In contrast, natural polymer-based microgels, such as alginate (Eydelnant et al., 2014; Miyama et al., 2013; Utech et al., 2015; Yu et al., 2015), agarose (Eydelnant et al., 2014; Shi et al., 2013) and chitosan (Jung et al., 2014), are widely used for cell encapsulation because of their biocompatibility and mild conditions required to achieve gelation, thus preserving cell viability. Cross-linking methods for gelation affect significantly the network structures of the formed microgels, so the ability to control cross-linking process allows the formation of homogeneous network structure with precise internal structure and tunable stiffness (Jung et al., 2014; Utech et al., 2015), enabling the stable entrapment of cells in a controlled microenvironment. Utech et al. prepared monodisperse, structurally homogeneous alginate microgels in the size range of 10–50 μm by the on-demand release of calcium ions from a water-soluble calcium–EDTA complex to initiate the ionic cross-linking (Utech et al., 2015). The ability to produce microgels with tunable size and high degree of uniformity is of key importance for cell encapsulation and cell culture within gel matrices, since cell–cell distances and structural arrangement have a significant influence on cellular properties and functions. Additionally, the degradability of microgels is essential for enhancing cell viability during long-term cell culture. Moreover, gel matrices can be fabricated to possess various hierarchical structures and any desired shape to

support cell culture, such as yarn-ball-shaped (Miyama et al., 2013), core-shell (Yu et al., 2015) or tubular (Jung et al., 2014). For example, Miyama et al. synthesized yarn-ball-shaped microgels which had an average outer diameter of 200 μm with fibers of 10–30 μm . They had a large void volume and a high surface-to-volume ratio, which provided sufficient permeability to promote effective delivery of oxygen, nutrients, and metabolic products to the encapsulated cells (Miyama et al., 2013). Yu et al. prepared alginate microgels having a structure of alginate-core/alginate-shell in which the core acted as 3D culturing unit while the shell prevented cells from diffusing out of the microgels and subsequently proliferate and form monolayer in the culture-flask as occurred to the core-only microgels (Yu et al., 2015).

3.2 3D cell culture for tumor-on-a-chip

Numerous types of nanoparticles (NPs) have been synthesized for pharmaceutical applications as previously described (*Section 2.2*). The delivery of these NPs to tumor sites is very complex. NPs can undergo multiple transport processes, such as blood flow-driven movement, NP–endothelium interactions and extravasation, and confront pathophysiological barriers, such as elevated interstitial pressure (Heldin et al., 2004). Recently, tumor-on-a-chip has emerged as a new system to evaluate the delivery of NPs, providing physiological relevance, organ-level function as well as enhanced throughput and reduced cost. A Tumor-on-a-chip model normally consists of tumor cells or spheroids representing tumor tissues and microchannels mimicking the blood flow.

To date, several tumor-on-a-chip platforms, including breast, lung, brain and liver tumor on-a-chip, have been established for various purposes (Table 2). Albanese et al. immobilized a multicellular melanoma tumor spheroid in a microfluidic device to evaluate the accumulation of gold NPs at tumors under physiological interstitial flows (Fig. 9) (Albanese et al., 2013). PEG-functionalized gold NPs having a smaller size (40 and 70 nm) accumulated at the tumor spheroid to a greater extent than the larger NPs (110 and 150 nm).

Functionalizing the NPs (40 nm) with a targeting molecule (i.e., iron-transporting transferrin protein (Tf)) further improved their accumulation and retention. Also, higher flow rates resulted in better accumulation but predominantly in the ECM around the spheroid. *In vivo* xenograft tumor model in mice further validated these results except the enhanced receptor targeting effect (Albanese et al., 2013). Prabhakarbandian et al. designed and fabricated a tumor-on-a-chip with a complex tumor-vascular network (Fig. 10A) (Prabhakarbandian et al., 2015), mimicking the morphology, fluidics and leaky vasculature of tumor microenvironment *in vivo*. Endothelial cells and 3D cervical tumor spheroids separated by the leaking gaps were co-cultured in the network (Fig. 10B) (Prabhakarbandian et al., 2015). The chip was used to predict *in vivo* delivery efficacy of two commercial nano polymer gene delivery vehicles (i.e., PPC and Express-In). The behavior of the delivery carriers (labeled with Rhodamine) under the flow of serum proteins were observed in the synthetic tumor network (Fig. 10C). PPC showed uniform distribution and minimal aggregation across the vascular and the tumor regions, whereas Express-In showed the opposite. Furthermore, the GFP expression using PPC and Express-In polymers injected into the tumor network were quantified using two different methods (direct and vascular) (Fig. 10D). Vascular injection showed higher efficiency for PPC while direct tumor injection showed similar results for both the polymers, although Express-In based-GFP expression signal was slightly higher. The results obtained from the synthetic tumor network were consistent with the *in vivo* delivery performance (Prabhakarbandian et al., 2015). Kwapiszewska et al. developed the SpheroChip, consisting of disposable microfluidic chips for cell culture and a positioning plate, to culture human cell lines (i.e., HT-29 colon cancer cells and Hep-G2 hepatocytes). After the injection of anticancer drug 5-fluorouracil (5-FU), the dynamic changes on the metabolic activity of the cells in response to the drug could be monitored and analyzed continuously (Kwapiszewska et al., 2014). Ruppen et al. observed a higher chemoresistance in primary co-culture

spheroids (i.e., primary lung cancer epithelial cells and primary pericytes) than in primary monoculture spheroids in a Lung-on-a-Chip model, which could serve as a more chemosensitive tumor model (Ruppen et al., 2015). More deep understanding about tumors is expected to be unveiled as the tumor-on-a-chip technique develops, which will contribute enormously to drug and nanocarrier designing, screening, and evaluation.

Fig. 9 Tumor-on-a-chip platform to study tissue transport behaviour of engineered gold nanoparticles (NPs). (A) Schematic illustration of the tumor-on-a-chip. (B) Optical view of the tumor-on-a-chip (scale bar, 1000 μm) (left) contained a spheroid (stained with anti-Laminin-fluorescein isothiocyanate) which was surrounded by a non-uniform layer of extracellular matrix (ECM) that acted as a physical barrier between the cells and the medium (scale bar, 100 μm) (right). (C, D) Localization (C) and kinetics (D) of tissue accumulation of Tf-functionalized NPs. The NPs predominately accumulated in the ECM surrounding the tumor spheroid (sphr) and that a targeting ligand (Tf) provided an “anchoring effect”, reducing efflux during flushing/washing. (E) Representative image of tumor fluorescence from *in vivo* mouse model injected with the Tf-functionalized NPs, showing that the result was largely in an agreement with those obtained using microfluidic *in vitro* model.

Reproduced with permission from (Albanese et al., 2013). Copyright (2013) Nature Publishing Group.

Fig. 10 Synthetic tumor network on chip. (A) Conceptual design of the synthetic tumor network on-chip consisting of vascular channels (for culturing endothelial cells) and tissue compartment (for culturing tumor) cells separated by walled barrier with the 2 μ m leaking gap to mimic “leaky vessel” in tumor microvasculature *in vivo*. (B) Co-culture of 3D HeLa cells (cultured on microfabricated scaffolds) and endothelial cells (cultured in the vascular

lumen) separated by the walled barrier (scale bar: 250 μ m). (C) Difference in the behavior of two commercial nanopolymer based gene delivery systems (labelled in Rhodamine fluorescent tag) in the synthetic tumor network (scale bars: 250 μ m). (D) Quantification of green fluorescent protein (GFP) intensity expressed in PPC and Express-In following vascular and direct injection. Reprinted from (Prabhakarpanthian et al., 2015), Copyright (2015), with permission from Elsevier.

Table 2 Representative examples of tumor-on-a-chip models and their applications.

Cancer model	Object	Applications	Ref.
Skin melanoma	–	Association between cancer and immune system	(Businaro et al., 2013)
Colon cancer	Anticancer drugs (e.g., 5-fluorouracil)	Anticancer efficacy; drug screening	(Kwapiszewska et al., 2014)
Breast cancer	–	Invasion	(Trietsch et al., 2013)
	Photosensitizer (e.g., 5-aminolevulinic acid) and gold nanoparticles	Photodynamic therapy evaluation	(Yang et al., 2015)
Lung cancer	Anticancer drugs (e.g., gefitinib and masitinib)	Anticancer efficacy; drug screening	(Aref et al., 2013)
	Cisplatin	Chemosensitivity evaluation	(Ruppen et al., 2015)
Brain glioma	Anticancer drugs (e.g., vincristine and bleomycin)	Therapeutic efficacy	(Liu et al., 2015b)
Liver cancer	–	Tumor immune surveillance	(Christakou et al., 2015)
Cervical cancer	Nanopolymers	Gene delivery	(Prabhakarpanthian et al., 2015)

3.3. 3D cell culture for organs-on-a-chip

So far, we have witnessed too many examples of new drugs that failed at late stages of their clinical trials, which is a heavy blow to the related pharmaceutical companies as well as public's confidence in fighting against devastating diseases such as cancer. The development of new drugs or new formulations has been extremely costly and time-consuming. The reason in relating to this is primarily due to the poor prediction capability of preclinical studies using existing preclinical models. Therefore, the importance of creating new models to generate reliable preclinical data of drug safety and efficacy has repeatedly been emphasized by the drug discovery community. Similar to tumor-on-a-chip (Section 3.2), organs can also be reconstructed in microfluidic devices to generate organs-on-a-chip systems. By applying 3D tissue culture or organoids in integrated microfluidic networks, such models can replicate the key functions of organs inside our body. Furthermore, the precise control over microfluidic flows offers advantages such as mimicking blood circulation, controlling over chemical and biomolecular gradients, and providing continuous medium exchange. Therefore, organs-on-a-chip models (Table 3) have great potential in mimicking the behavior and efficacy of drugs/nanoparticles more accurately than conventional 2D cell cultures, much faster and less expensive than *in vivo* animal models.

Table 3 Recently reported single and multiple organ-on-chip systems and their applications.

Organ(s)	Object	Applications	Ref.
Lung	Nanoparticles and interleukin-2	Inflammation, toxicity	(Huh et al., 2012; Huh et al., 2010)
Liver	Antipyretic analgesics	Drug metabolism	(Mao et al., 2012)
Bone marrow	Antimetabolite chemotherapeutic	Acute lymphoblastic leukemia (ALL)	(Bruce et al., 2015)
Blood vessels	—	Vascular function	(Kim et al., 2015b;

			Wang et al., 2014b)
Kidney	Cisplatin	Toxicity	(Jang et al., 2013)
Brain	–	Brain function, Alzheimer's disease	(Berdichevsky et al., 2010; Park et al., 2015)
Heart	–	Heart function	(Yazdi et al., 2015)
Intestine, liver, tumor	Anticancer drug (e.g., cyclophosphamide and tegafur)	Drug metabolism, anticancer activity	(Imura et al., 2012)
Liver, bone marrow, tumor	Anticancer drug (e.g., 5-fluorouracil)	Drug metabolism, toxicity	(Sung et al., 2010)
Intestine, liver, skin, kidney	–	Absorption, distribution, metabolism and excretion (ADME)	(Maschmeyer et al., 2015)
Liver, brain tumor	Anticancer drug (e.g. temozolomide and ifosfamide)	Anticancer activity	(Ma et al., 2012)
Liver, skin	Troglitazone	Toxicity	(Wagner et al., 2013)

Lung-on-a-chip was the first trial of the concept of organ-on-a-chip. A layer of human alveolar epithelial cells and a layer of human pulmonary microvascular endothelial cells were co-cultured on the opposite sides of a thin porous elastomeric membrane to simulate the alveolar–capillary interface (Huh et al., 2010). This microfluidic design enabled the application of differential media, shear stress, and a mechanical stretch–shrink movement over the cells simultaneously to mimic the physiological breathing motions (Figs. 11A, B). Exposing the epithelial side to 20 nm-diameter NPs (Fig. 11C) resulted in the toxic and inflammatory response as indicated by translocation of the NPs across the alveolar–capillary interface as well as increased reactive oxygen species (ROS) production and intercellular

adhesion molecule-1 (ICAM-1) expression. More importantly, the NP translocation was significantly augmented by applying the cyclic mechanical lateral stretching, an effect that could not be obtained using conventional Transwell cultures (Fig. 11D). A whole-mouse-lung ventilation-perfusion model was then used to determine the physiological relevance of this observation. After the intra-tracheal injection into the breathing mouse lung, the 20-nm NPs reached the surface of alveolar epithelium and the underlying interstitial space and microvasculature (Fig. 11E), and the NP transport from the alveoli into the microvasculature was significantly increased in the presence of cyclic breathing *in vivo* (Fig. 11F), similar to those observed in the lung mimic device *in vitro* (Fig. 11D). This lung-on-a-chip model was also utilized to investigate the toxicity of an anticancer drug interleukin-2 (IL-2) aiming to discover new drug candidates (Huh et al., 2012). In another study, the brain was grafted onto a microchip, and axons formed between the cortex and the hippocampus. Moreover, the selective pharmacological manipulation of activities in the constituent slices was demonstrated, which could help us to understand the development, plasticity, and pathologies of neural systems. Other organs such as bone marrow and kidney have also been successfully “transplanted” into microfluidic systems to establish microengineered models for various applications (Table 3). These systems could be of great support in obtaining preclinical data such as toxicity and maximum tolerated doses.

Fig. 11 Lung-on-a-chip. (A) Schematic of on-chip cell culture model providing microenvironment mechanical stimuli for reconstituting organ-level function of lung. (B) Photograph of the actual lung-on-a-chip device. (C) Transport of nanoparticles (NPs) from the alveolar chamber to the vascular channel of the lung-on-a-chip, simulating transport across the alveolar-capillary interface of the lung. (D) Toxicological test of 20-nm NPs using

the lung-on-a-chip: 10% mechanical strain was applied to simulate breathing motions compared with static controls in this device or in a Transwell culture system. (E–F) Validation of the toxicity of the 20-nm NPs using the whole mouse lung: fluorescence micrographs of a histological section of the lung showing the NPs locations (E) and the number of NPs absorbed into the blood perfusate over time with and without physiological cyclic breathing through application of mechanical ventilation in the lung (F). PC, pulmonary capillary; AS, alveolar space; blue, epithelial nucleus; scale bar, 20 μm . Reprinted from (Huh et al., 2010) with permission from The American Association for the Advancement of Science.

Apart from single organ models, multiple organs on a chip (organs-on-a-chip) have also been established by biologically connecting different tissues or organs to study their collective effects (Table 3). For example, chambers containing intestine, liver, and breast cancer cells were connected in series to investigate the absorption, metabolism, and activity of an anticancer drug after oral delivery (Fig. 12) (Imura et al., 2012). In another study, a colon tumor, liver, and bone marrow were combined to explore the *in vivo* metabolism and cytotoxicity of the anticancer drug 5-fluorouracil (5-FU) (Sung et al., 2010). Similarly, Ma et al. reconstituted liver and brain tumor on a microchip, and found that the activation of ifosfamide by the liver led to enhanced brain tumor cytotoxicity (Ma et al., 2012). These findings may provide important primary pharmacokinetic and pharmacodynamic (PK/PD) data for discovering novel drugs. Although we have not yet seen a successful precedent of any organ-on-chip model for the commercial development of novel drugs, this technology has gradually been incorporated into various fundamental research and hold great promise in accelerating the development of novel drugs and drug delivery carriers.

Fig. 12 Organs-on-a-chip. Cross-sectional illustration (drawn not to scale) of the multi-organ model representing stomach digestion, intestine absorption and liver metabolism before an anticancer drug reaching tumor site after oral delivery. Reprinted from (Imura et al., 2012) with permission from The Japan Society for Analytical Chemistry.

4. Perspective

Convergence of microfluidics and material fabrication provides an attractive strategy for making micro- and nanomaterials with controlled properties for pharmaceutical applications. The flexibility of tuning the properties of these materials precisely such as size, surface charge, surface morphology, hierarchical structure, etc., and the possibility of producing them in a reproducible and scalable manner have led researchers to develop a plethora of various micro- and nanomaterials exhibiting unique properties and promising functions, and the research focus has moved gradually from the initial efforts in developing different microfluidic devices and strategies in precisely controlling micro- and nanosystems' physical properties, such as size, monodispersity, shape, surface charge, and texture, to recent attempts in fabricating micro- and nanosystems with more complex structures, such as core-shell, Janus and nano-in-micro, carrying multi-components, such as two or more drugs,

quantum dots and MRI agents. These engineered micro- and nanosystems often can achieve multiple functions, including triggered release by pH, temperature, light, and even external fields such as ultrasound, heating, etc., targeted delivery by integrating targeting ligands or external magnetic force, and real-time therapeutics and imaging for theranostics. Although the advance in this field is fast, the translation of these micro- and nanosystems has been slow due to two main obstacles. One is that most of the appealing properties such as complex structures and multifunctions will lose their advantages that have been demonstrated in *in vitro* models once they are introduced to the real *in vivo* systems and ultimately in human. Specifically, more complexities and functions mean more convoluted behavior and effects *in vivo*, thus making it more difficult in understanding the interactions between these micro- and nanosystems and the biological systems at all levels from cell through to tissue and organs. On the other hand, the lack of quick, cheap and reliable models represents another obstacle to understand these complicated interactions.

The transition from 2D to 3D models is a critical step towards more physiologically relevant tissue models. However, traditional 3D culture techniques suffer from several limitations. For example, they did not capture the multicellular complexity of tissues, were lack of vasculature, cannot provide control over gradients, and cannot offer medium exchange in a continuous manner. Microfluidics can overcome all of these limitations by co-culturing cells in a spatially controlled manner, generating and controlling gradients, and integrating continuous perfusion and flow. Therefore, significant research interest has been attracted in developing various microfluidic 3D cell culture models, tumor-on-a-chip, organs-on-a-chip or even human body-on-a-chip. After a decade of intensive research, considerable progress has been achieved in this field, and the bottle neck for developing microfluidic models has shifted from design of different microfluidic model systems to their validation

and implementation. As the initial intent was to develop models that better mimic human biology, animal models, despite their wide use, are thus not suitable for validation. Ideally, the microfluidic models can be validated using available clinical data. However, there are not much data available publically. Alternatively, relevant biological functions or biological biomarkers can be used for validation. In this respect, the challenge would be using analytical tools that are sensitive enough to be able to detect the biomarkers over a range of concentrations from a small amount of samples. Additionally, to better mimic human biology, primary cells from human are necessary for forming 3D cultures. However, there are several challenges for using primary cells such as hard to culture, short life span in vitro, batch-to-batch variation. Stem cell techniques, especially induced pluripotent stem cells (iPSc) and the progress in controlling differentiation of the stem cells could provide a solution. Integration of stem cells or organoids with microfluidic models could revolutionise current development of microfluidic-based models. Although it is still at a very early stage of their development, the advent of organs-on-a-chip platforms will surely provide valuable inputs in exploring the complex interactions, thus deepening our understanding of the ultimate requirement of the design of micro- and nanosystems for real pharmaceutical applications.

Acknowledgements

The authors acknowledge the research funding by the Australian Research Council (ARC) under Future Fellowship Project (FT140100726) and Discovery Project (DP150100798). C.-X.Z. acknowledges financial support from the award of the ARC Future Fellowship (FT140100726). R.R. and T.B. acknowledge PhD scholarships from the University of Queensland.

References

- Abbaspourrad, A., Carroll, N.J., Kim, S.H., Weitz, D.A., 2013a. Polymer microcapsules with programmable active release. *J. Am. Chem. Soc.* 135, 7744–7750.
- Abbaspourrad, A., Datta, S.S., Weitz, D.A., 2013b. Controlling release from pH-responsive microcapsules. *Langmuir* 29, 12697–12702.
- Abbaspourrad, A., Duncanson, W.J., Lebedeva, N., Kim, S.H., Zhushma, A.P., Datta, S.S., Dayton, P.A., Sheiko, S.S., Rubinstein, M., Weitz, D.A., 2013c. Microfluidic fabrication of stable gas-filled microcapsules for acoustic contrast enhancement. *Langmuir* 29, 12352–12357.
- Albanese, A., Lam, A.K., Sykes, E.A., Rocheleau, J.V., Chan, W.C.W., 2013. Tumour-on-a-chip provides an optical window into nanoparticle tissue transport. *Nat. Commun.* 4, 1–8.
- An, H.Z., Safai, E.R., Eral, H.B., Doyle, P.S., 2013. Synthesis of biomimetic oxygen-carrying compartmentalized microparticles using flow lithography. *Lab Chip* 13, 4765–4774.
- Anton, N., Bally, F., Serra, C.A., Ali, A., Arntz, Y., Mely, Y., Zhao, M.J., Marchioni, E., Jakhmola, A., Vandamme, T.F., 2012. A new microfluidic setup for precise control of the polymer nanoprecipitation process and lipophilic drug encapsulation. *Soft Matter* 8, 10628–10635.
- Aref, A.R., Huang, R.Y.-J., Yu, W., Chua, K.-N., Sun, W., Tu, T.-Y., Bai, J., Sim, W.-J., Zervantonakis, I.K., Thiery, J.P., Kamm, R.D., 2013. Screening therapeutic EMT blocking agents in a three-dimensional microenvironment. *Integr. Biol.* 5, 381–389.
- Baah, D., Tigner, J., Bean, K., Walker, N., Britton, B., Floyd-Smith, T., 2012. Microfluidic synthesis and post processing of non-spherical polymeric microparticles. *Microfluid. Nanofluid.* 12, 657–662.
- Balbino, T.A., Aoki, N.T., Gasperini, A.A.M., Oliveira, C.L.P., Azzoni, A.R., Cavalcanti, L.P., de la Torre, L.G., 2013. Continuous flow production of cationic liposomes at high lipid

concentration in microfluidic devices for gene delivery applications. Chem. Eng. J. 226, 423–433.

Belliveau, N.M., Huft, J., Lin, P.J.C., Chen, S., Leung, A.K.K., Leaver, T.J., Wild, A.W., Lee, J.B., Taylor, R.J., Tam, Y.K., Hansen, C.L., Cullis, P.R., 2012. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for *in vivo* delivery of siRNA. Mol. Ther. Nucleic Acids 1, e37.

Berdichevsky, Y., Staley, K.J., Yarmush, M.L., 2010. Building and manipulating neural pathways with microfluidics. Lab Chip 10, 999–1004.

Bokharai, M., Schneider, T., Dutz, S., Stone, R.C., Mefford, O.T., Häfeli, U.O., 2016. Production of monodispersed magnetic polymeric microspheres in a microfluidic chip and 3D simulation. Microfluid. Nanofluid. 20, 1–14.

Bruce, A., Evans, R., Mezan, R., Shi, L., Moses, B.S., Martin, K.H., Gibson, L.F., Yang, Y., 2015. Three-dimensional microfluidic tri-culture model of the bone marrow microenvironment for study of acute lymphoblastic leukemia. Plos One 10, e0146203.

Businaro, L., De Ninno, A., Schiavoni, G., Lucarini, V., Ciasca, G., Gerardino, A., Belardelli, F., Gabriele, L., Mattei, F., 2013. Cross talk between cancer and immune cells: exploring complex dynamics in a microfluidic environment. Lab Chip 13, 229–239.

Capretto, L., Carugo, D., Cheng, W., Hill, M., Zhang, X.L., 2011. Continuous-flow production of polymeric micelles in microreactors: experimental and computational analysis. J. Colloid Interface Sci. 357, 243–251.

Cetin, B., Asik, M.D., Taze, S., 2014. Design and fabrication of a microfluidic device for synthesis of chitosan nanoparticles. J. Nanotechnol. Eng. Med. 4, 031004.

Chan, J.M., Zhang, L., Yuet, K.P., Liao, G., Rhee, J.-W., Langer, R., Farokhzad, O.C., 2009. PLGA–lecithin–PEG core–shell nanoparticles for controlled drug delivery. Biomaterials 30, 1627–1634.

- Chen, B., Deng, J., Tong, L., Yang, W., 2010. Optically active helical polyacetylene@silica hybrid organic–inorganic core/shell nanoparticles: preparation and application for enantioselective crystallization. *Macromolecules* 43, 9613–9619.
- Chen, D., Love, K.T., Chen, Y., Eltoukhy, A.A., Kastrup, C., Sahay, G., Jeon, A., Dong, Y., Whitehead, K.A., Anderson, D.G., 2012. Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation. *J. Am. Chem. Soc.* 134, 6948–6951.
- Chen, Q.L., Liu, Z., Shum, H.C., 2014a. Three-dimensional printing-based electro-millifluidic devices for fabricating multi-compartment particles. *Biomicrofluidics* 8, 064112.
- Chen, S., Zhang, H., Shi, X., Wu, H., Hanagata, N., 2014b. Microfluidic generation of chitosan/CpG oligodeoxynucleotide nanoparticles with enhanced cellular uptake and immunostimulatory properties. *Lab Chip* 14, 1842–1849.
- Choi, C.H., Kang, S.M., Jin, S.H., Yi, H., Lee, C.S., 2015a. Controlled fabrication of multicompartamental polymeric microparticles by sequential micromolding via surface-tension-induced droplet formation. *Langmuir* 31, 1328–1335.
- Choi, C.H., Lee, B., Kim, J., Nam, J.O., Yi, H., Lee, C.S., 2015b. Controlled fabrication of microparticles with complex 3D geometries by tunable interfacial deformation of confined polymeric fluids in 2D micromolds. *ACS Appl. Mater. Interfaces* 7, 11393–11401.
- Christakou, A.E., Ohlin, M., Onfelt, B., Wiklund, M., 2015. Ultrasonic three-dimensional on-chip cell culture for dynamic studies of tumor immune surveillance by natural killer cells. *Lab Chip* 15, 3222–3231.
- Dash, M., Chiellini, F., Ottenbrite, R.M., Chiellini, E., 2011. Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Prog. Polym. Sci.* 36, 981–1014.

- Dong, H., Tang, G., Ma, T., Cao, X., 2015. One-step fabrication of inorganic/organic hybrid microspheres with tunable surface texture for controlled drug release application. *J. Mater. Sci. Mater. Med.* 27, 1–8.
- Duarte, A.R.C., Unal, B., Mano, J.F., Reis, R.L., Jensen, K.F., 2014. Microfluidic production of perfluorocarbon-alginate core-shell microparticles for ultrasound therapeutic applications. *Langmuir* 30, 12391–12399.
- Duncanson, W.J., Lin, T., Abate, A.R., Seiffert, S., Shah, R.K., Weitz, D.A., 2012a. Microfluidic synthesis of advanced microparticles for encapsulation and controlled release. *Lab Chip* 12, 2135–2145.
- Duncanson, W.J., Zieringer, M., Wagner, O., Wilking, J.N., Abbaspourrad, A., Haag, R., Weitz, D.A., 2012b. Microfluidic synthesis of monodisperse porous microspheres with size-tunable pores. *Soft Matter* 8, 10636–10640.
- Ekanem, E.E., Nabavi, S.A., Vladisavljevic, G.T., Gu, S., 2015. Structured biodegradable polymeric microparticles for drug delivery produced using flow focusing glass microfluidic devices. *ACS Appl. Mater. Interfaces* 7, 23132–23143.
- Elsayed, M., Huang, J., Edirisinghe, M., 2015. Bioinspired preparation of alginate nanoparticles using microbubble bursting. *Mater. Sci. Eng., C* 46, 132–139.
- Eydelnant, I.A., Li, B.B., Wheeler, A.R., 2014. Microgels on-demand. *Nat. Commun.* 5, 1–9.
- Fan, J.B., Huang, C., Jiang, L., Wang, S.T., 2013. Nanoporous microspheres: from controllable synthesis to healthcare applications. *J. Mater. Chem. B* 1, 2222–2235.
- Fang, R.H., Aryal, S., Hu, C.-M.J., Zhang, L., 2010. Quick synthesis of lipid-polymer hybrid nanoparticles with low polydispersity using a single-step sonication method. *Langmuir* 26, 16958–16962.

- Fang, R.H., Chen, K.N.H., Aryal, S., Hu, C.-M.J., Zhang, K., Zhang, L., 2012. Large-scale synthesis of lipid–polymer hybrid nanoparticles using a multi-inlet vortex reactor. *Langmuir* 28, 13824–13829.
- Ferreira, D.S., Reis, R.L., Azevedo, H.S., 2013. Peptide-based microcapsules obtained by self-assembly and microfluidics as controlled environments for cell culture. *Soft Matter* 9, 9237–9248.
- Foster, T., Dorfman, K.D., Davis, H.T., 2010. Giant biocompatible and biodegradable PEG-PMCL vesicles and microcapsules by solvent evaporation from double emulsion droplets. *J. Colloid Interface Sci.* 351, 140–150.
- Funakoshi, K., Suzuki, H., Takeuchi, S., 2007. Formation of giant lipid vesiclelike compartments from a planar lipid membrane by a pulsed jet flow. *J. Am. Chem. Soc.* 129, 12608–12609.
- Gañán-Calvo, A.M., Montanero, J.M., Martín-Banderas, L., Flores-Mosquera, M., 2013. Building functional materials for health care and pharmacy from microfluidic principles and Flow Focusing. *Adv. Drug Deliv. Rev.* 65, 1447–1469.
- Ge, X.H., Huang, J.P., Xu, J.H., Luo, G.S., 2014. Controlled stimulation-burst targeted release by smart decentered core-shell microcapsules in gravity and magnetic field. *Lab Chip* 14, 4451–4454.
- Gokmen, M.T., De Geest, B.G., Hennink, W.E., Du Prez, F.E., 2009. "Giant" hollow multilayer capsules by microfluidic templating. *ACS Appl. Mater. Interfaces* 1, 1196–1202.
- Gref, R., Domb, A., Quellec, P., Blunk, T., Muller, R.H., Verbavatz, J.M., Langer, R., 2012. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Adv. Drug Deliv. Rev.* 64, 316–326.
- Günther, A., Jensen, K.F., 2007. Multiphase microfluidics: from flow characteristics to chemical and materials synthesis. *Lab Chip* 7, 399–399.

- Guo, Z.X., Zhang, M., Zhao, L.B., Guo, S.S., Zhao, X.Z., 2011. Generation of alginate gel particles with AuNPs layers by polydimethylsiloxan template. *Biomicrofluidics* 5, 026502.
- Hadinoto, K., Sundaresan, A., Cheow, W.S., 2013. Lipid–polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review. *Eur. J. Pharm. Biopharm.* 85, 427–443.
- Hayakawa, M., Onoe, H., Nagai, K.H., Takinoue, M., 2016. Complex-shaped three-dimensional multi-compartmental microparticles generated by diffusional and Marangoni microflows in centrifugally discharged droplets. *Sci. Rep.* 6, 20793.
- Heldin, C.-H., Rubin, K., Pietras, K., Ostman, A., 2004. High interstitial fluid pressure – an obstacle in cancer therapy. *Nat. Rev. Cancer* 4, 806–813.
- Herranz-Blanco, B., Liu, D.F., Makila, E., Shahbazi, M.A., Ginestar, E., Zhang, H.B., Aseyev, V., Balasubramanian, V., Salonen, J., Hirvonen, J., Santos, H.A., 2015. On-chip self-assembly of a smart hybrid nanocomposite for antitumoral applications. *Adv. Funct. Mater.* 25, 1488–1497.
- Hines, D.J., Kaplan, D.L., 2013. Poly(lactic-co-glycolic acid)-controlled-release systems: experimental and modeling insights. *Crit. Rev. Ther. Drug Carrier Syst.* 30, 257–276.
- Hong, J.S., Stavis, S.M., Lacerda, S.H.D., Locascio, L.E., Raghavan, S.R., Gaitan, M., 2010. Microfluidic directed self-assembly of liposome–hydrogel hybrid nanoparticles. *Langmuir* 26, 11581–11588.
- Hood, R.R., DeVoe, D.L., 2015. High-throughput continuous flow production of nanoscale liposomes by microfluidic vertical flow focusing. *Small* 11, 5790–5799.
- Hood, R.R., Shao, C.R., Omiatsek, D.M., Vreeland, W.N., DeVoe, D.L., 2013. Microfluidic synthesis of PEG- and folate-conjugated liposomes for one-step formation of targeted stealth nanocarriers. *Pharm. Res.* 30, 1597–1607.

- Hood, R.R., Vreeland, W.N., DeVoe, D.L., 2014. Microfluidic remote loading for rapid single-step liposomal drug preparation. *Lab Chip* 14, 3359–3367.
- Hu, Y.D., Wang, Q., Wang, J.Y., Zhu, J.T., Wang, H., Yang, Y.J., 2012. Shape controllable microgel particles prepared by microfluidic combining external ionic crosslinking. *Biomicrofluidics* 6, 026502.
- Huang, K.S., Yang, C.H., Kung, C.P., Grumezescu, A.M., Ker, M.D., Lin, Y.S., Wang, C.Y., 2014. Synthesis of uniform core-shell gelatin-alginate microparticles as intestine-released oral delivery drug carrier. *Electrophoresis* 35, 330–336.
- Huh, D., Leslie, D.C., Matthews, B.D., Fraser, J.P., Jurek, S., Hamilton, G.A., Thorne, K.S., McAlexander, M.A., Ingber, D.E., 2012. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. *Sci. Transl. Med.* 4, 159ra147.
- Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., Ingber, D.E., 2010. Reconstituting organ-level lung functions on a chip. *Science* 328, 1662–1668.
- Hung, L.H., Teh, S.Y., Jester, J., Lee, A.P., 2010. PLGA micro/nanosphere synthesis by droplet microfluidic solvent evaporation and extraction approaches. *Lab Chip* 10, 1820–1825.
- Imura, Y., Yoshimura, E., Sato, K., 2012. Micro total bioassay system for oral drugs: evaluation of gastrointestinal degradation, intestinal absorption, hepatic metabolism, and bioactivity. *Anal. Sci.* 28, 197–197.
- Jagadeesan, D., Nasimova, I., Gourevich, I., Starodubtsev, S., Kumacheva, E., 2011. Microgels for the encapsulation and stimulus-responsive release of molecules with distinct polarities. *Macromol. Biosci.* 11, 889–896.
- Jahn, A., Lucas, F., Wepf, R.A., Dittrich, P.S., 2013. Freezing continuous-flow self-assembly in a microfluidic device: toward imaging of liposome formation. *Langmuir* 29, 1717–1723.
- Jahn, A., Stavis, S.M., Hong, J.S., Vreeland, W.N., DeVoe, D.L., Gaitan, M., 2010. Microfluidic mixing and the formation of nanoscale lipid vesicles. *ACS Nano* 4, 2077–2087.

- Jahn, A., Vreeland, W.N., Gaitan, M., Locascio, L.E., 2004. Controlled vesicle self-assembly in microfluidic channels with hydrodynamic focusing. *J. Am. Chem. Soc.* 126, 2674–2675.
- Jang, K.-J., Mehr, A.P., Hamilton, G.A., McPartlin, L.A., Chung, S., Suh, K.-Y., Ingber, D.E., 2013. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. *Integr. Biol.* 5, 1119–1129.
- Jung, J., Kim, K., Choi, S.C., Oh, J., 2014. Microfluidics-assisted rapid generation of tubular cell-laden microgel inside glass capillaries. *Biotechnol. Lett.* 36, 1549–1554.
- Kamat, V., Marathe, I., Ghormade, V., Bodas, D., Paknikar, K., 2015. Synthesis of monodisperse chitosan nanoparticles and in situ drug loading using active microreactor. *ACS Appl. Mater. Interfaces* 7, 22839–22847.
- Karnik, R., Gu, F., Basto, P., Cannizzaro, C., Dean, L., Kyei-Manu, W., Langer, R., Farokhzad, O.C., 2008. Microfluidic platform for controlled synthesis of polymeric nanoparticles. *Nano Lett.* 8, 2906–2912.
- Kastner, E., Verma, V., Lowry, D., Perrie, Y., 2015. Microfluidic-controlled manufacture of liposomes for the solubilisation of a poorly water soluble drug. *Int. J. Pharm.* 485, 122–130.
- Khan, I.U., Serra, C.A., Anton, N., Er-Rafik, M., Blanck, C., Schmutz, M., Kraus, I., Messaddeq, N., Sutter, C., Anton, H., Klymchenko, A.S., Vandamme, T.F., 2015a. Microfluidic conceived Trojan microcarriers for oral delivery of nanoparticles. *Int. J. Pharm.* 493, 7–15.
- Khan, I.U., Serra, C.A., Anton, N., Li, X., Akasov, R., Messaddeq, N., Kraus, I., Vandamme, T.F., 2014. Microfluidic conceived drug loaded Janus particles in side-by-side capillaries device. *Int. J. Pharm.* 473, 239–249.
- Khan, I.U., Serra, C.A., Anton, N., Vandamme, T., 2013. Microfluidics: a focus on improved cancer targeted drug delivery systems. *J. Control. Release* 172, 1065–1074.

- Khan, I.U., Serra, C.A., Anton, N., Vandamme, T.F., 2015b. Production of nanoparticle drug delivery systems with microfluidics tools. *Expert Opin. Drug Deliv.* 12, 547–562.
- Kim, C., Kang, J.Y., 2014. Microfluidic device for continuous observation of cellular behaviors in specialized core-shell microcapsules. *Biochip J.* 8, 199–208.
- Kim, D.H., Chen, J., Omary, R.A., Larson, A.C., 2015a. MRI visible drug eluting magnetic microspheres for transcatheter intra-arterial delivery to liver tumors. *Theranostics* 5, 477–488.
- Kim, J.-W., Utada, A.S., Fernández-Nieves, A., Hu, Z., Weitz, D.A., 2007. Fabrication of monodisperse gel shells and functional microgels in microfluidic devices. *Angew. Chem. Int. Ed.* 46, 1819–1822.
- Kim, J., Chung, M., Kim, S., Jo, D.H., Kim, J.H., Jeon, N.L., 2015b. Engineering of a biomimetic pericyte-covered 3D microvascular network. *Plos One* 10, e0133880.
- Kim, K., Kang, D.H., Kim, M.S., Kim, K.S., Park, K.M., Hong, S.C., Chang, P.S., Jung, H.S., 2015c. Generation of alginate nanoparticles through microfluidics-aided polyelectrolyte complexation. *Colloids Surf. A, Physicochem. Eng. Asp.* 471, 86–92.
- Kim, Y., Lee Chung, B., Ma, M., Mulder, W.J.M., Fayad, Z.A., Farokhzad, O.C., Langer, R., 2012. Mass production and size control of lipid–polymer hybrid nanoparticles through controlled microvortices. *Nano Lett.* 12, 3587–3591.
- Kolishetti, N., Dhar, S., Valencia, P.M., Lin, L.Q., Karnik, R., Lippard, S.J., Langer, R., Farokhzad, O.C., 2010. Engineering of self-assembled nanoparticle platform for precisely controlled combination drug therapy. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17939–17944.
- Kong, F., Zhang, X., Zhang, H.B., Qu, X.M., Chen, D., Servos, M., Makila, E., Salonen, J., Santos, H.A., Hai, M.T., Weitz, D.A., 2015. Inhibition of multidrug resistance of cancer cells by co-delivery of DNA nanostructures and drugs using porous silicon nanoparticles@giant liposomes. *Adv. Funct. Mater.* 25, 3330–3340.

- Kong, T.T., Wu, J., To, M., Yeung, K.W.K., Shum, H.C., Wang, L.Q., 2012. Droplet based microfluidic fabrication of designer microparticles for encapsulation applications. *Biomicrofluidics* 6, 034104.
- Koynova, R., Tenchov, B., 2015. Recent progress in liposome production, relevance to drug delivery and nanomedicine. *Recent Pat. Nanotechnol.* 9, 86–93.
- Kurmi, B.D., Kayat, J., Gajbhiye, V., Tekade, R.K., Jain, N.K., 2010. Micro- and nanocarrier-mediated lung targeting. *Expert Opin. Drug Deliv.* 7, 781–794.
- Kwapiszewska, K., Michalczyk, A., Rybka, M., Kwapiszewski, R., Brzozka, Z., 2014. A microfluidic-based platform for tumour spheroid culture, monitoring and drug screening. *Lab Chip* 14, 2096–2104.
- Leon, R.A.L., Somasundar, A., Badruddoza, A.Z.M., Khan, S.A., 2015. Microfluidic fabrication of multi-drug-loaded polymeric microparticles for topical glaucoma therapy. *Part. Part. Syst. Char.* 32, 567–572.
- Leong, W., Wang, D.-A., 2015. Cell-laden polymeric microspheres for biomedical applications. *Trends Biotechnol.* 33, 653–666.
- Li, W.X., Dong, H., Tang, G.N., Ma, T., Cao, X.D., 2015. Controllable microfluidic fabrication of Janus and microcapsule particles for drug delivery applications. *RSC Adv.* 5, 23181–23188.
- Liao, C.Y., Su, Y.C., 2010. Formation of biodegradable microcapsules utilizing 3D, selectively surface-modified PDMS microfluidic devices. *Biomed. Microdevices* 12, 125–133.
- Lim, J.M., Bertrand, N., Valencia, P.M., Rhee, M., Langer, R., Jon, S., Farokhzad, O.C., Karnik, R., 2014. Parallel microfluidic synthesis of size-tunable polymeric nanoparticles using 3D flow focusing towards in vivo study. *Nanomed. Nanotech. Biol. Med.* 10, 401–409.

- Liu, H., Qian, X., Wu, Z., Yang, R., Sun, S., Ma, H., 2016a. Microfluidic synthesis of QD-encoded PEGDA microspheres for suspension assay. *J. Mater. Chem. B* 4, 482–488.
- Liu, M., Sun, X.-T., Yang, C.-G., Xu, Z.-R., 2016b. On-chip preparation of calcium alginate particles based on droplet templates formed by using a centrifugal microfluidic technique. *J. Colloid Interface Sci.* 466, 20–27.
- Liu, W., Chen, X.D., Selomulya, C., 2015a. On the spray drying of uniform functional microparticles. *Particuology* 22, 1–12.
- Liu, W.J., Chen, X.D., Cheng, Z.N., Selornulya, C., 2016c. On enhancing the solubility of curcumin by microencapsulation in whey protein isolate via spray drying. *J. Food Eng.* 169, 189–195.
- Liu, W.J., Selomulya, C., Chen, X.D., 2013. Design of polymeric microparticles for pH-responsive and time-sustained drug release. *Biochem. Eng. J.* 81, 177–186.
- Liu, W.J., Wu, W.D., Selomulya, C., Chen, X.D., 2012. Spray drying of monodispersed microencapsulates: implications of formulation and process parameters on microstructural properties and controlled release functionality. *J. Microencapsul.* 29, 677–684.
- Liu, W.M., Xu, J., Li, T.B., Zhao, L., Ma, C., Shen, S.F., Wang, J.Y., 2015b. Monitoring tumor response to anticancer drugs using stable three-dimensional culture in a recyclable microfluidic platform. *Anal. Chem.* 87, 9752–9760.
- Liu, Y., Li, K., Pan, J., Liu, B., Feng, S.-S., 2010. Folic acid conjugated nanoparticles of mixed lipid monolayer shell and biodegradable polymer core for targeted delivery of Docetaxel. *Biomaterials* 31, 330–338.
- Liu, Y.M., Wu, W., Ju, X.J., Wang, W., Xie, R., Mou, C.L., Zheng, W.C., Liu, Z., Chu, L.Y., 2014. Smart microcapsules for direction-specific burst release of hydrophobic drugs. *RSC Adv.* 4, 46568–46575.

- Lu, B., Tarn, M.D., Pamme, N., Georgiou, T.K., 2015. Tailoring pH-responsive acrylic acid microgels with hydrophobic crosslinks for drug release. *J. Mater. Chem. B* 3, 4524–4529.
- Luo, R.C., Cao, Y., Shi, P., Chen, C.H., 2014. Near-infrared light responsive multi-compartmental hydrogel particles synthesized through droplets assembly induced by superhydrophobic surface. *Small* 10, 4886–4894.
- Ma, L., Barker, J., Zhou, C., Li, W., Zhang, J., Lin, B., Foltz, G., Küblbeck, J., Honkakoski, P., 2012. Towards personalized medicine with a three-dimensional micro-scale perfusion-based two-chamber tissue model system. *Biomaterials* 33, 4353–4361.
- Majedi, F.S., Hasani-Sadrabadi, M.M., VanDersarl, J.J., Mokarram, N., Hojjati-Emami, S., Dashtimoghadam, E., Bonakdar, S., Shokrgozar, M.A., Bertsch, A., Renaud, P., 2014. On-chip fabrication of paclitaxel-loaded chitosan nanoparticles for cancer therapeutics. *Adv. Funct. Mater.* 24, 432–441.
- Makadia, H.K., Siegel, S.J., 2011. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 3, 1377.
- Mao, S., Gao, D., Liu, W., Wei, H., Lin, J.-M., 2012. Imitation of drug metabolism in human liver and cytotoxicity assay using a microfluidic device coupled to mass spectrometric detection. *Lab Chip* 12, 219–226.
- Maschmeyer, I., Lorenz, A.K., Schimek, K., Hasenberg, T., Ramme, A.P., Hubner, J., Lindner, M., Drewell, C., Bauer, S., Thomas, A., Sambo, N.S., Sonntag, F., Lauster, R., Marx, U., 2015. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip* 15, 2688–2699.
- McGuigan, A.P., Sefton, M.V., 2007. Design and fabrication of sub-mm-sized modules containing encapsulated cells for modular tissue engineering. *Tissue Eng.* 13, 1069–1078.

- Mendes, A.C., Baran, E.T., Lisboa, P., Reis, R.L., Azevedo, H.S., 2012. Microfluidic fabrication of self-assembled peptide-polysaccharide microcapsules as 3D environments for cell culture. *Biomacromolecules* 13, 4039–4048.
- Mieszawska, A.J., Kim, Y., Gianella, A., van Rooy, I., Priem, B., Labarre, M.P., Ozcan, C., Cormode, D.P., Petrov, A., Langer, R., Farokhzad, O.C., Fayad, Z.A., Mulder, W.J.M., 2013. Synthesis of polymer–lipid nanoparticles for image-guided delivery of dual modality therapy. *Bioconjug. Chem.* 24, 1429–1434.
- Mijajlovic, M., Wright, D., Zivkovic, V., Bi, J.X., Biggs, M.J., 2013. Microfluidic hydrodynamic focusing based synthesis of POPC liposomes for model biological systems. *Colloids Surf. B, Biointerfaces* 104, 276–281.
- Min, N.G., Ku, M., Yang, J., Kim, S.-H., 2016. Microfluidic production of uniform microcarriers with multicompartments through phase separation in emulsion drops. *Chem. Mater.* 28, 1430–1438.
- Miyama, A., Yamada, M., Sugaya, S., Seki, M., 2013. A droplet-based microfluidic process to produce yarn-ball-shaped hydrogel microbeads. *RSC Adv.* 3, 12299–12306.
- Oh, J., Kim, K., Choi, S., Jung, J., 2014. Diffusion-assisted spherical microgel fabrication using in situ gelable chitosan and dextran. *Dig. J. Nanomater. Bios.* 9, 739–744.
- Paques, J.P., van der Linden, E., van Rijn, C.J.M., Sagis, L.M.C., 2014. Preparation methods of alginate nanoparticles. *Adv. Colloid Interface Sci.* 209, 163–171.
- Park, J., Byun, A., Kim, D.H., Shin, S.S., Kim, J.H., Kim, J.W., 2014. Microfluidic fabrication and permeation behaviors of uniform zwitterionic hydrogel microparticles and shells. *J. Colloid Interface Sci.* 426, 162–169.
- Park, J., Lee, B.K., Jeong, G.S., Hyun, J.K., Lee, C.J., Lee, S.H., 2015. Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an in vitro model of Alzheimer's disease. *Lab Chip* 15, 141–150.

- Park, J.H., Saravanakumar, G., Kim, K., Kwon, I.C., 2010. Targeted delivery of low molecular drugs using chitosan and its derivatives. *Adv. Drug Deliv. Rev.* 62, 28–41.
- Peer, D., Karp, J.M., Hong, S., Farokhzad, O.C., Margalit, R., Langer, R., 2007. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* 2, 751–760.
- Pessi, J., Santos, H.A., Miroshnyk, I., Yliruusi, J., Weitz, D.A., Mirza, S., 2014. Microfluidics-assisted engineering of polymeric microcapsules with high encapsulation efficiency for protein drug delivery. *Int. J. Pharm.* 472, 82–87.
- Phapal, S.M., Sunthar, P., 2013. Influence of micro-mixing on the size of liposomes self-assembled from miscible liquid phases. *Chem. Phys. Lipids* 172–173, 20–30.
- Prabaharan, M., 2015. Chitosan-based nanoparticles for tumor-targeted drug delivery. *Int. J. Biol. Macromolec.* 72, 1313–1322.
- Prabhakarapandian, B., Shen, M.C., Nichols, J.B., Garson, C.J., Mills, I.R., Matar, M.M., Fewell, J.G., Pant, K., 2015. Synthetic tumor networks for screening drug delivery systems. *J. Control. Release* 201, 49–55.
- Qiang, F., Jin, S., Wenping, Z., Xiaofan, S., Zhongtian, Y., Zhonggui, H., 2009. Nanoparticle albumin-bound (NAB) technology is a promising method for anti-cancer drug delivery. *Recent Pat. Anticancer Drug Discov.* 4, 262–272.
- Ran, R., Middelberg, A.P.J., Zhao, C.X., 2016. Microfluidic synthesis of multifunctional liposomes for tumour targeting. *Colloid Surface B* 148, 402–410.
- Rhee, M., Valencia, P.M., Rodriguez, M.I., Langer, R., Farokhzad, O.C., Karnik, R., 2011. Synthesis of size-tunable polymeric nanoparticles enabled by 3D hydrodynamic flow focusing in single-layer microchannels. *Adv. Mater.* 23, H79–H83.
- Riahi, R., Tamayol, A., Shaegh, S.A.M., Ghaemmaghami, A.M., Dokmeci, M.R., Khademhosseini, A., 2015. Microfluidics for advanced drug delivery systems. *Curr. Opin. Chem. Eng.* 7, 101–112.

- Ruppen, J., Wildhaber, F.D., Strub, C., Hall, S.R.R., Schmid, R.A., Geiser, T., Guenat, O.T., 2015. Towards personalized medicine: chemosensitivity assays of patient lung cancer cell spheroids in a perfused microfluidic platform. *Lab Chip* 15, 3076–3085.
- Ryan, T.D., Donghwan, K., Jaesung, P., 2012. Formation of liposomes using a 3D flow focusing microfluidic device with spatially patterned wettability by corona discharge. *J. Micromech. Microeng.* 22, 055003.
- Schroeder, A., Levins, C.G., Cortez, C., Langer, R., Anderson, D.G., 2010. Lipid-based nanotherapeutics for siRNA delivery. *J. Intern. Med.* 267, 9–21.
- Seiffert, S., 2012. Non-spherical soft supraparticles from microgel building blocks. *Macromol. Rapid Commun.* 33, 1286–1293.
- Seiffert, S., Weitz, D.A., 2010. Microfluidic fabrication of smart microgels from macromolecular precursors. *Polymer* 51, 5883–5889.
- Shi, Y., Gao, X.H., Chen, L.Q., Zhang, M., Ma, J.Y., Zhang, X.X., Qin, J.H., 2013. High throughput generation and trapping of individual agarose microgel using microfluidic approach. *Microfluid. Nanofluid.* 15, 467–474.
- Shim, T.S., Kim, S.H., Yang, S.M., 2013. Elaborate design strategies toward novel microcarriers for controlled encapsulation and release. *Part. Part. Syst. Char.* 30, 9–45.
- Skorb, E.V., Möhwald, H., 2014. “Smart” surface capsules for delivery devices. *Adv. Mater. Interf.* 1, 1400237.
- Stirland, D.L., Nichols, J.W., Miura, S., Bae, Y.H., 2013. Mind the gap: a survey of how cancer drug carriers are susceptible to the gap between research and practice. *J. Control. Release* 172, 1045–1064.
- Sun, J.S., Xianyu, Y.L., Li, M.M., Liu, W.W., Zhang, L., Liu, D.B., Liu, C., Hu, G.Q., Jiang, X.Y., 2013. A microfluidic origami chip for synthesis of functionalized polymeric nanoparticles. *Nanoscale* 5, 5262–5265.

- Sun, J.S., Zhang, L., Wang, J.L., Feng, Q., Liu, D.B., Yin, Q.F., Xu, D.Y., Wei, Y.J., Ding, B.Q., Shi, X.H., Jiang, X.Y., 2015. Tunable rigidity of (polymeric core)–(lipid shell) nanoparticles for regulated cellular uptake. *Adv. Mater.* 27, 1402–1407.
- Sung, J.H., Kam, C., Shuler, M.L., 2010. A microfluidic device for a pharmacokinetic-pharmacodynamic (PK-PD) model on a chip. *Lab Chip* 10, 446–455.
- Trietsch, S.J., Israels, G.D., Joore, J., Hankemeier, T., Vulto, P., 2013. Microfluidic titer plate for stratified 3D cell culture. *Lab Chip* 13, 3548–3554.
- Tsuda, Y., Morimoto, Y., Takeuchi, S., 2010. Monodisperse cell-encapsulating peptide microgel beads for 3D cell culture. *Langmuir* 26, 2645–2649.
- Utech, S., Prodanovic, R., Mao, A.S., Ostafe, R., Mooney, D.J., Weitz, D.A., 2015. Microfluidic generation of monodisperse, structurally homogeneous alginate microgels for cell encapsulation and 3D cell culture. *Adv. Healthc. Mater.* 4, 1628–1633.
- Valencia, P.M., Basto, P.A., Zhang, L.F., Rhee, M., Langer, R., Farokhzad, O.C., Karnik, R., 2010. Single-step assembly of homogenous lipid–polymeric and lipid–quantum dot nanoparticles enabled by microfluidic rapid mixing. *Acs Nano* 4, 1671–1679.
- Valencia, P.M., Pridgen, E.M., Rhee, M., Langer, R., Farokhzad, O.C., Karnik, R., 2013. Microfluidic platform for combinatorial synthesis and optimization of targeted nanoparticles for cancer therapy. *Acs Nano* 7, 10671–10680.
- van Swaay, D., deMello, A., 2013. Microfluidic methods for forming liposomes. *Lab Chip* 13, 752–767.
- Vasiliauskas, R., Liu, D.F., Cito, S., Zhang, H.B., Shahbazi, M.A., Sikanen, T., Mazutis, L., Santos, H.A., 2015. Simple microfluidic approach to fabricate monodisperse hollow microparticles for multidrug delivery. *ACS Appl. Mater. Interfaces* 7, 14822–14832.
- Velasco, D., Tumarkin, E., Kumacheva, E., 2012. Microfluidic encapsulation of cells in polymer microgels. *Small* 8, 1633–1642.

- Vladisavljevic, G.T., Khalid, N., Neves, M.A., Kuroiwa, T., Nakajima, M., Uemura, K., Ichikawa, S., Kobayashi, I., 2013. Industrial lab-on-a-chip: design, applications and scale-up for drug discovery and delivery. *Adv. Drug Deliv. Rev.* 65, 1626–1663.
- Wagner, I., Materne, E.-M., Brincker, S., Su, Fradrich, C., Busek, M., Sonntag, F., Sakharov, D.A., Trushkin, E.V., Tonevitsky, A.G., Lauster, R., Marx, U., 2013. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. *Lab Chip* 13, 3538–3547.
- Wang, A.Z., Langer, R., Farokhzad, O.C., 2012. Nanoparticle delivery of cancer drugs, in: Caskey, C.T., Austin, C.P., Hoxie, J.A. (Eds.), *Ann. Rev. Med.*, pp. 185–198.
- Wang, W., Zhang, M.J., Chu, L.Y., 2014a. Functional polymeric microparticles engineered from controllable microfluidic emulsions. *Acc. Chem. Res.* 47, 373–384.
- Wang, X.Y., Jin, Z.H., Gan, B.W., Lv, S.W., Xie, M., Huang, W.H., 2014b. Engineering interconnected 3D vascular networks in hydrogels using molded sodium alginate lattice as the sacrificial template. *Lab Chip* 14, 2709–2716.
- Wong, H.L., Rauth, A.M., Bendayan, R., Manias, J.L., Ramaswamy, M., Liu, Z., Erhan, S.Z., Wu, X.Y., 2006. A new polymer–lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. *Pharm. Res.* 23, 1574–1585.
- Wu, J., Kong, T.T., Yeung, K.W.K., Shum, H.C., Cheung, K.M.C., Wang, L.Q., To, M.K.T., 2013. Fabrication and characterization of monodisperse PLGA-alginate core-shell microspheres with monodisperse size and homogeneous shells for controlled drug release. *Acta Biomater.* 9, 7410–7419.
- Xu, X., Appel, E.A., Liu, X., Parker, R.M., Scherman, O.A., Abell, C., 2015. Formation of cucurbit[8]uril-based supramolecular hydrogel beads using droplet-based microfluidics. *Biomacromolecules* 16, 2743–2749.

- Xue, P., Wu, Y.F., Menon, N.V., Kang, Y.J., 2015. Microfluidic synthesis of monodisperse PEGDA microbeads for sustained release of 5-fluorouracil. *Microfluid. Nanofluid.* 18, 333–342.
- Yang, C.H., Huang, K.S., Lin, Y.S., Lu, K., Tzeng, C.C., Wang, E.C., Lin, C.H., Hsu, W.Y., Chang, J.Y., 2009. Microfluidic assisted synthesis of multi-functional polycaprolactone microcapsules: incorporation of CdTe quantum dots, Fe₃O₄ superparamagnetic nanoparticles and tamoxifen anticancer drugs. *Lab Chip* 9, 961–965.
- Yang, C.H., Wang, C.Y., Grumezescu, A.M., Wang, A.H.J., Hsiao, C.J., Chen, Z.Y., Huang, K.S., 2014. Core-shell structure microcapsules with dual pH-responsive drug release function. *Electrophoresis* 35, 2673–2680.
- Yang, Y., Yang, X., Zou, J., Jia, C., Hu, Y., Du, H., Wang, H., 2015. Evaluation of photodynamic therapy efficiency using an in vitro three-dimensional microfluidic breast cancer tissue model. *Lab Chip* 15, 735–744.
- Yazdi, S.R., Shadmani, A., Burgel, S.C., Misun, P.M., Hierlemann, A., Frey, O., 2015. Adding the 'heart' to hanging drop networks for microphysiological multi-tissue experiments. *Lab Chip* 15, 4138–4147.
- Yeh, C.H., Chen, K.R., Lin, Y.C., 2013. Developing heatable microfluidic chip to generate gelatin emulsions and microcapsules. *Microfluid. Nanofluid.* 15, 775–784.
- Yeh, C.H., Chen, Y.C., Lin, Y.C., 2011. Generation of droplets with different concentrations using gradient-microfluidic droplet generator. *Microfluid. Nanofluid.* 11, 245–253.
- Yoon, D.H., Hasegawa, K., Kaneko, Y., Arakawa, T., Go, J.S., Sekiguchi, T., Shoji, S., 2015. Formation of polymeric hollow microcapsules and microlenses using gas-in-organic-in-water droplets. *Micromachines-Basel* 6, 622–633.

Yu, L.F., Ni, C., Grist, S.M., Bayly, C., Cheung, K.C., 2015. Alginate core-shell beads for simplified three-dimensional tumor spheroid culture and drug screening. *Biomed. Microdevices* 17.

Yu, Y.L., Zhang, M.J., Xie, R., Ju, X.J., Wang, J.Y., Pi, S.W., Chu, L.Y., 2012. Thermo-responsive monodisperse core-shell microspheres with PNIPAM core and biocompatible porous ethyl cellulose shell embedded with PNIPAM gates. *J. Colloid Interface Sci.* 376, 97–106.

Zhang, H.B., Liu, D.F., Shahbazi, M.A., Makila, E., Herranz-Blanco, B., Salonen, J., Hirvonen, J., Santos, H.A., 2014. Fabrication of a multifunctional nano-in-micro drug delivery platform by microfluidic templated encapsulation of porous silicon in polymer matrix. *Adv. Mater.* 26, 4497–4503.

Zhang, L., Chan, J.M., Gu, F.X., Rhee, J.-W., Wang, A.Z., Radovic-Moreno, A.F., Alexis, F., Langer, R., Farokhzad, O.C., 2008. Self-assembled lipid-polymer hybrid nanoparticles: a robust drug delivery platform. *Acs Nano* 2, 1696–1702.

Zhang, L., Feng, Q., Wang, J., Zhang, S., Ding, B., Wei, Y., Dong, M., Ryu, J.-Y., Yoon, T.-Y., Shi, X., Sun, J., Jiang, X., 2015. Microfluidic synthesis of hybrid nanoparticles with controlled lipid layers: understanding flexibility-regulated cell-nanoparticle interaction. *Acs Nano* 9, 9912–9921.

Zhang, M.J., Wang, W., Xie, R., Ju, X.J., Liu, L., Gu, Y.Y., Chu, L.Y., 2013. Microfluidic fabrication of monodisperse microcapsules for glucose-response at physiological temperature. *Soft Matter* 9, 4150–4159.

Zhao, C.X., 2013. Multiphase flow microfluidics for the production of single or multiple emulsions for drug delivery. *Adv. Drug Deliv. Rev.* 65, 1420–1446.

Zhao, C.X., He, L.Z., Qiao, S.Z., Middelberg, A.P.J., 2011. Nanoparticle synthesis in microreactors. *Chem. Eng. Sci.* 66, 1463–1479.

Zhao, C.X., Middelberg, A.P.J., 2011. Two-phase microfluidic flows. *Chem. Eng. Sci.* 66, 1394–1411.

Zieringer, M.A., Carroll, N.J., Abbaspourrad, A., Koehler, S.A., Weitz, D.A., 2015. Microcapsules for enhanced cargo retention and diversity. *Small* 11, 2903–2909.

Highlights

- Multiphase microfluidics for the synthesis of micro-/nanostructures is presented.
- Development of carriers with multiple functionalities and components is discussed.
- Utilization of microfluidics towards developing biomimetic chips is highlighted.
- Special emphasis is on tumor-on-a-chip and organs-on-a-chip for carriers evaluation.