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Microfluidics technology for drug delivery: A review

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1. ABSTRACT

Microfluidics is undoubtedly an influential technology that is currently revolutionizing the chemical and biological studies by replicating laboratory bench-top technology on a miniature chip-scale device. In the area of drug delivery science, microfluidics offers advantages, such as precise dosage, ideal delivery, target-precise delivery, sustainable and controlled release, multiple dosing, and slight side effects. These advantages bring significant assets to the drug delivery systems. Microfluidic technology has been progressively used for fabrication of drug carriers, direct drug delivery systems, high-throughput screening, and formulation and immobilization of drugs. This review discusses the recent technological progress, outcomes and available opportunities for the usage of microfluidics systems in drug delivery systems.

2. INTRODUCTION

Nowadays, new drug developments and drug delivery approaches have attracted considerable attention, and most of the studies in this area of science have focused on increasing the bioaccessibility and bioavailability of the drugs, decreasing the side effects of the drugs, and improving the comfort and satisfaction of the patients (1).

The conventional delivery methods, such as oral, sublingual, rectal, intravenous, subcutaneous, and intramuscular drug deliveries have disadvantages, such as low solubility and permeability, degradation by the enzymes, interactions with foods, probable toxicity due to the incorrect drug dosage, inflammation, and irregular absorption (2). Nevertheless, new technologies, like microfluidics are potent to solve these

problems. Latest developments in microfabrication have opened the opportunity to control the microfluidic systems that may apply for biological systems. Indeed, microfluidics in comparison to the other traditional fabricated devices has lower costs for fabrication, and it offers better functionality and reliability combined with different miniature devices. Additionally, the miniaturized usage of components and the accurate control of the volume of fluid in microfluidics reduce the waste products, and it enables accurate temperature control and rapid temperature exchange (3). Evidence demonstrate that, the microfluidic systems have a great potential to be used in a vast area of applications, such as chemical screening, cell culture, cell separation and one single cell manipulation, electrophoretic separations, and DNA sequencing (4). The microfluidics drug delivery systems can be used for the proteins, peptides, and the DNA-based drugs that are vulnerable to enzymatic degradation through the long pathway of current drug delivery systems (5). The portable miniaturized microfluidic devices allow mobile applications in chemical analysis and in producing drug on demand. Also, microfluidic systems have the potential to provide an efficient microarray platform for the fabrication of a range of pharmaceutical materials in micro and Nano-scale (6). At the Micro and Nano-scale, the surface-based phenomena become more significant than volume-based phenomena and the viscous resistance of the laminar flow. Additionally, the convective transport and mixing are more difficult on these scales. Therefore, the mass transport depends mostly on molecular diffusion (7). Considerably, these characteristics of microfluidic systems are valuable for studying the drug delivery systems, since the size of the drugs and the cells are at the scale of nanometers and micrometer, respectively. Recently, significant progress has been stated on using microfluidics for the drug delivery, for example, the possibility of the control and the release of the bioactive molecule “dexamethasone” from microfibers of a hyaluronic acid amphiphilic derivative obtained by microfluidic through changing the salt concentration have been reported (8). Also, a cheap, reproducible and effective technique based on microfluidic technology for the synthesis of vinylidene fluoride-co-chlorotrifluoroethylene microstructures with tailored architecture, morphology, and wettability is reported by Cardoso *et al.* (9).

Microfluidics can be used for the synthesis of the polymers with precise forms or chemistries that have applications in tissue engineering, barcoding, diagnostic imaging, and drug delivery. An example is the strategy of using the capillary instability-driven breakup of a liquid jet formed by two immiscible fluids to produce polymer particles with different shapes and morphologies developed by Nie *et al.* (10). The described strategy allows precise control of the emulsification process, leading to the production of monodispersed droplets with controlled morphologies in a size range from 20 to 200 µm.

In addition, microfluidic systems can be applied for screening the process and synthesizing the homogenous particles for the delivery of the drugs. In this regard, Wu *et al.* used a microfluidic device to study the interactions between the microstructures and profile of the drug release by synthesis of the monodisperse poly (lactic-co-glycolic acid) microspheres at the varied diameters sizes (11). They found out dissimilarities in the expansion rates with small and large poly (lactic-co-glycolic acid) microspheres have homogeneous and heterogeneous drug release characteristics, respectively. Moreover, monodisperse particles (polydispersity index=3.9%, defined as the ratio between the standard deviation and the mean diameter of particles multiplied by 100) in a defined size range from 10µm to 50 µm were fabricated by Xu *et al.* using a microfluidic device (12). Kinetic analysis of the monodisperse particles showed that, the release of the drug is slower than that of conventional methods of the same average size. The difference in the initial kinetics of drug release was attributed to the uniform distribution of the drug inside the particles generated by the microfluidic device. Metz *et al.* also used the implantable and flexible polyimide probes with microelectrodes and embedded microfluidic channels for simultaneous, selective chemical delivery drug delivery and multi-channel monitoring of bioelectric activities (13).

All of these evidence shows that microfluidics technologies have the potential to be used as a game changer in drug delivery systems. The objective of this study is to discuss the recent technological progress, outcomes and available opportunities for the usage of microfluidics systems in drug delivery systems.

3. FABRICATION OF DRUG DELIVERY CARRIERS

Drug delivery systems (DDS) require a uniform and well-shaped capsule for the immobilization of drugs into microparticles (MP) and nanoparticles (NP). Different authors have summarized methodologies used to produce NPs (14,15). However, several techniques involve in the usage of reagents with a significant level of toxicity or biological hazards that suppose a risk to the environment (16). Different challenges using NPs for drug encapsulation have been addressed, emphasizing those associated with physical characteristics, such as size distribution, non-uniform shape, and encapsulation efficiencies (17). For this reason, the techniques to produce MPs or NPs are the key factor to obtain identical, uniform and homogenous particles to enhance drug encapsulation. In this regard, microfluidics as a multidisciplinary engineering field with well-established techniques may represent an alternative to aid most of the MPs and NPs problems regarding their physical characteristics. The development of an effective drug carrier means

fabricating a material that improves bioavailability, stabilizes reduces the side effects and releases the drug in a controlled manner. For example, oral delivery carriers should resist the acidic conditions of the stomach to be released in the small intestine and pass through the intestinal membrane to the bloodstream.

Microfluidic devices allow the fabrication of uniform particles, such as microparticles, microcapsules, and microgels via single or multiple emulsions as templates (18). For example, recently non-ionic surfactant based vesicles (niosomes) that are self-assembled synthetic membrane from a mixture of cholesterol and diacetyl phosphate was fabricated via microfluidics. Also, a microfluidic device was designed that allows the rapid mixing of two miscible reagents in microchannels to obtain niosomes 40% smaller than those produced by the bulk method (19). Another device was fabricated to produce synthetic nanoparticles by controlling the precipitation of polymers via manipulation of the flow in the microfluidic channels to produce nanoparticles from PLGA-PEG and PLGA (20).

One of the main advantages of using this technology in DDS is even though that, the small amounts of drugs and reagents are needed, but enhancements in drug encapsulation, and productions of uniform particles with lower variation coefficient (<5%) are observable (21,22). This higher efficiency of the drug encapsulation is because of the active component is mixed with the dispersed phase, which will later become automatically encapsulated in the process. Tailor-made nano and microparticles can be achieved by changing different parameters, such as fluid phase flow rate, equipment geometry, and liquid properties, such as Reynolds number, capillary number, and viscosity (19,22). Several studies have found out that microfluidics systems enhances the ratio of loading drug into the particle (23), and it can allow the encapsulation of multiple drugs in one particle even if these drugs have a hydrophobic or hydrophilic nature (24,25).

Materials and the geometry are two main parameters for fabrication of a microfluidic device. Different materials have been used for fabrication of microfluidics devices, includeing polydimethylsiloxane (PDMS) (26,27), poly(methyl methacrylate) (PMMA) (28,29), polycarbonate (PC) (30), cyclic olefin copolymer (COC) (31,32), polyetheretherketone (PEEK) (33), polyimide plastic resin (PIPR) (13,34), glass (35,36), quartzose (37,38), and silicon (39). Also, the combination of two materials, such as PMMA and PC (40) or PDMS and PC (41) were used by the researchers. Some of the most common geometries for microfluidics devices are T-junction (42,43), co-flowing (44), and flow-focusing (45,46). In the case of T-junction geometries, two immiscible fluids are joined by an intersection where two channels converge forming a "T" shape. The resulting mixing of both phases directs the flow in one direction to the end of the device. The co-flowing geometry directs both phases in the same orientation. A microchannel is immersed in another microchannel, where the dispersed phase is placed in the inner channel, while the continuous phase is placed between the outer, inner channel and inside the outer channel. In flow focusing geometries, both phases are in contrary directions until they intersect at the end of the inner channel. The dispersed phase enters the inner channel while the continuous phase streams between outer and inner channels and enters the inner channel too. Despite the basic configurations, a microfluidic device with multiple geometries can be designed to obtain a desired effect on the droplets. Continuous T-junction intersections can create different droplets conformations or combining T-junctions followed by a flow focusing geometry.

3.1. Single and multiple emulsion in microfluidics

Single emulsions are formed by mixing of two immiscible liquids where a dispersed phase is placed into a continuous phase. Particles are produced due to the convergence of both phases in an intersection of the device. Each phase can be either a hydrophilic solution or hydrophobic one. The nature of the particle will be manipulated by selection of the solution for dispersed or continuous phase. Water-in-oil (W/O) droplets can be generated when water is used as a continuous phase, and it is used for hydrophobic drugs. Oil-in-water (O/W) droplets are generated when oil is used as a dispersed phase, and water is placed as a continuous phase. As previously mentioned, droplet morphology, size, and shape controlled by changing specific parameters, such as flow rate, the geometry of the device, and dimensionless parameters (Reynolds and capillary number). A multiple emulsion can be defined as an emulsion in an emulsion. The nature of the phase will determine which type of emulsion will be. Multiple emulsions consist of several drops immersed into a second phase, and then the past mixture is immersed into a third phase. The most common multiple emulsions consist of a water-in-oil-in-water ($W_1/O/W_2$) and oil-in-water-in-oil ($O_1/W/O_2$). However, other solvents can be used based on the nature of the phase (i.e. water can be exchanged for any aqueous or polar phase while oil can be exchanged for any hydrophobic solution). Depending on the number of the phases employed, emulsions can be classified as double, triple, and quadruple, and so on. From all the multiple emulsions that can be formed, double emulsions are preferred because they simplify the production of tailor-made droplets with the desired size, structure and composition, and allow to use those droplets as a template of other microcapsules (47). In comparison with single emulsions, double emulsions allow the encapsulation of polar and non-polar molecules, and it can enhance the controlled

Table 1. Drug encapsulation in PLA and PLGA for different applications

Drug	Description	Applications	Encapsulation Material	Ref.
Camptothecin	Topoisomerase I inhibitor	Anti-cancer: Colon Cancer	PLA; PLGA	58
Bupivacaine	Local anesthetic	Local infiltration, peripheral nerve block	PLGA	12
Rifampicin	Inhibits bacterial RNA polymerase	Anti-mycobacterium: tuberculosis, leprosy	PLGA	60
Haloperidol	Dopamine antagonist	Psychiatry: mood disorders, schizoaffective disorders	PLGA	60
Insulin	Therapeutic protein	Diabetes Mellitus 1 and 2	Chitosan	61

release because of the additional barrier produced with the middle phase enhancing the flexibility of the droplet formed (22). Multiple emulsions are usually designed to encapsulate food additives or drugs for pharmaceutical purposes (48,49).

3.2. Carrier materials

Different materials can be used to produce micro and nanoparticles (50). The main characteristics of a suitable material are biodegradability, biocompatibility, and non-toxicity. In this regard, mesoporous materials provide systems with a uniform porous surface that can be easily functionalized with other materials, such as iron oxide, gold, silver, etc. Different mesoporous materials have been used to encapsulate drugs, proteins or bioactive compounds, such as MCM-41 (51), MCF (52) and SBA-15 (53). Lately, magnetic particles have gained attention due to their ability to target specific cells in the body without being an invasive treatment. Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most common materials used to functionalize a particle with magnetic characteristics additionally to the low toxicity and stability that they present (50). Metallic materials, such as gold, aluminum, platinum, silver, and titanium dioxide are also used for that purpose (54). However, polymeric materials are widely for their biocompatibility, low toxicity, biodegradability, and sustained controlled release over long periods. Polymers can be modified to achieve a better response in physiological environments (55). In microfluidics, polymers, such as poly(vinyl alcohol) (PVA) (23), polylactide (PLA) and its family of copolymers, including poly(lactic-co-glycolic acid) (PLGA) (56,57), poly(ethylene glycol) (PEG) (23), and poly(caprolactone) (PCL) (23) are being used to immobilize bioactive compounds to study their feasibility as a drug delivery carriers. For example, camptothecin, an anticancer drug, has been encapsulated into PLA and PLGA matrices. These matrices were selected microencapsulated because they have the most attractive fluidic structure in pharmaceutics since it gathers certain characteristics needed by this industry, such as immobilization process (e.g. cells, proteins or enzymes), protection against degradation, and drug-delivery control (58). Table 1 presents some studies related to drug encapsulation in PLA and PLGA matrixes. To manufacture the droplets, Ito *et al.* used Shirasu Porous Glass (SPG) membranes (58). Their results showed the particles obtained of 2–3 μm in diameter had higher encapsulation efficiency in PLA than in PLGA, the same result was observed with drug release. Amphiphilic drugs have been encapsulated as well in PLGA matrix, for example, Xu *et al.* encapsulated bupivacaine (12). Also, microencapsulation was performed with PLGA containing rifampicin a drug used to treat tuberculosis. This microencapsulation was obtained by microchannel emulsification-solvent evaporation method. Haloperidol, a dopamine antagonist, used in psychiatry is also an example of microencapsulation made with PLGA, which has been performed with a modified membrane encapsulation process called Cross Flow Membrane Emulsification (XME) (60).

Membrane emulsification technique is used in combination with a cross-linking method to encapsulate peptides and proteins. Wang *et al.* prepared chitosan microspheres containing insulin and they obtained a high efficiency and maintained its chemical stability (61). The examples mentioned have demonstrated that microencapsulation can be versatile and its process is modifiable to help maintain the solubility and bioavailability of the target drug.

4. DRUG FORMULATION

The candidate drug has to be evaluated for toxicological and pharmacological effects both using *in vitro* and *in vivo* assays. In the case of toxicological studies, the cell-cell interactions can be mimicked with a microfluidic system using a network of interconnected chambers, with each chamber representing an organ. The effects of combinations of drugs can also be tested with these systems (62). Preclinical assessment of cytotoxicity using organ-in-a-chip systems can replicate *in vivo* conditions (3). During a clinical trial, pharmacokinetic (PK) and pharmacodynamics (PD) profiles for the drug in humans have to be established. The PK profile measures absorption, distribution, metabolism, and elimination of the drug, while the PD profile measures physiological and biological response to the drug. Blood sampling and processing are key for determining both of these profiles (62).

Drugs can be encapsulated to be protected from degradation via enzymes or to decrease toxicity and liposomes can be used to encapsulate

Table 2. Different microfluidics systems and carrier materials used for drug delivery

Material used	Geometry	Carrier material	Drug	Application	Ref.
Glass	Co-flowing	Human serum albumin	Doxorubicin	Hepatic cancer	44
PEEK and silica tube	T junction	Poly(methyl acrylate) and poly(acrylamide)	Ketoprofen and ranitidine	Suppression of gastric irritation effects: bleeding and ulceration	24
Silicon	Flow focusing	PLGA	Ciclosporin	-	57
PDMS	Co-flowing with herringbone shape	Liposomes	Propofol	Anesthetic agent	102
Quartz	Chip	Hyaluronic acid and ethylenediamine	Dexamethasone	Cell differentiation of mesenchymal stem cells	8
Glass	Co-flowing and flow-focusing	Polycaprolactone, poly(vinyl alcohol) and poly(ethylene glycol)	Bovine serum albumin	Protein therapy	23
PDMS	T junction	Poly(ethylene glycol) diacrylate	5-fluorouracil	Cancer therapy	103
PDMS	Flow focusing	Echogenic liposomes	Recombinant tissue-plasminogen activator (rt-PA)	Ischemic stroke	64
PMMA	V-junction	Poly(methylsilsesquioxane)	Itraconazole	Antifungal drug for infections	104

drugs and reduce their toxicity. In this regard, a microfluidic channel was fabricated to formulate liposome-encapsulated drugs by using a stream of lipids dissolved in alcohol divided into two sheathed streams in a microfluidic channel. The particle size could be changed from 50 to 150 nm by adjusting the ratio of alcohol/aqueous flow rate (62). Phospholipid nanoparticles were prepared using microfluidic technology, and the resulting liposomes were compared to those formed via the more commonly used thin film hydration method. There were no negative effects on the encapsulation efficiency, and overall microfluidic liposome preparation was a more simple, faster process compared to the thin film hydration technique (63). Protein microcapsules were developed via the emulsion technique using a water-oil-water system in a microfluidic device. The encapsulation efficiency was estimated to be 84%, and the obtained particles were stable for up to 4 weeks. These results suggest that this encapsulation system could be potentially applied for the drug delivery (23).

5. IMMOBILIZATION OF DRUGS USING MICROFLUIDICS FOR DRUG DELIVERY

In the case of drug delivery systems, the immobilization or drug loading into a particle using microfluidic systems begins with the mixing of the drug with one of the phases. Depending on the number of phases used, the encapsulation of the drug can be achieved using either single or multiple emulsions. The particle formation occurs when the drug mixed into the dispersed phase is passed through the microchannel and contacts a second phase (continuous phase). The shear rate caused by the fluid and the contact with walls of the microchannel allows the formation of the particle. This self-assembly reaction is achieved through a process called hydrodynamic flow focusing (HFF) (3). Usually, hydrophobic drugs are loaded using oil-in-water (O/W) phases, while hydrophilic drugs are loaded using water-in-oil (W/O) phases. Table 2 presents some applications of drug delivery carriers produced with microfluidic devices.

Bains *et al.* studied the effect of water content of the dye (as a fluorescent probe) 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate (DiI) into a poly(ε-caprolactone)-block-poly(ethylene oxide) (PCL-*b*-PEO) nanoparticle produced in a gas-liquid segmented microfluidic device. The designed geometry consisted of sinusoidal mixing channel of 100μm wide and 100mm in length, while the whole channel had a size of 200μm wide and 740 mm in length with a channel depth of 150 μm. The encapsulation of DiI loaded PCL-b-PEO nanoparticles involved the injection of 4 phases in the microfluidic device: (1) argon gas (Ar), (2) solution of PCL-b-PEO in dimethylformamide (DMF) with DiI dissolved, (3) water and DMF, and (4) DMF. The procedure followed allowed the formation of small spheres, lamellae and long filaments with a size of 70 nm, 800 nm, and 2000 nm respectively at a flow rate of 25 μL/min. Interestingly, filaments were not produced when the flow rate was increased at the double. However, filaments were produced again when a third increment of the flow rate at the double was done, meaning that the shape and size of the particles produced are dependent on the flow rate. Regarding the loading efficacies at the different water, concentrations were found out that at high water content (75%) the loading efficiency was 53% while at lower water concentrations (10%) had a higher loading efficiency 94%. Drug release was found to be faster at low water content, and a sustained and longer release was observed at higher water content (26).

Another interesting study in this area is the production of microbeads using microfluidics to encapsulate doxorubicin (DOXO) in human serum albumin (HAS) microparticles for the transcatheter arterial chemoembolization (TACE) of hepatic cancer (44). In this study, the material used for fabrication of the microfluidic device was glass using a co-flowing geometry. The used tube had an inner diameter of 0.5.8 mm and an outer diameter of 1 mm. The glass tube was tapered in one of the ends by heating with a final diameter from 200 to 250 µm. The single emulsion (W/O) used was formed by an oil phase (MCT oil) with 1% (v/v/v) of Span 80 and glutaraldehyde (solidifying agent), and the aqueous phase contained HSA solution with 0.2.% (w/v) DOXO and Tween 80. The flow rate used for the aqueous phase and the continuous phases were 3 mL/h and 10 mL/h respectively. The solidification of the particles was achieved by chemical crosslinking of glutaraldehyde with HSA. The particle size was obtained in an average of 183 µm when a ratio of the oil phase and the aqueous phase was 30:1. The decrement of the oil phase flow (5:1) increased the particle size almost at double of size (351 µm). It is very clear that one of the main parameters for particle size is the flow rate of at least one of the phases. *In vitro* results showed a fast release of the drug in the first 24 h of 10% of the total loaded drug, but after that, the release was sustained in the following 30 days with a total release of 16%. Regarding the *in vivo* results, this technique helped to inhibit tumor growth and DOXO-HSA particles were observed in the tissue after 24 days of the injection. TACE technique resulted in being more efficient than free DOXO injection in the tumor.

A microfluidic device allows the encapsulation of multiple drugs in the same droplet. An example is the Khan *et al.* studies on the encapsulation of ketoprofen and ranitidine HCl into a core-shell microparticle (24). In this case, ketoprofen was loaded into the core composed of poly (methyl acrylate), while ranitidine HCl was loaded into the shell composed of poly (acrylamide). The average size of the particles produced ranged between 100 to 151 µm. Two T-geometry were used in the microfluidic device, which was manufactured with an internal tube of PEEK and an external tube of PTFE (polytetrafluoroethylene). Silicon oil was used as a continuous phase, and the dispersed phases were ranitidine-shell and ketoprofen-core. After the T-junction, UV irradiation was applied to polymerize the particles using 704 mW/cm^2 irradiation. In this specific case, the flow rate of the continuous phase and middle phase (Q_c/Q_m) played an important role in the formation of the particles because when this ratio was increased, the particles produced were smaller. The encapsulation efficiency for ketoprofen was higher than ranitidine HCl with more than 80% and 50% respectively. Drug release of ranitidine HCl was faster than ketoprofen, and it had a fast release of both drugs in the first 8 h, while a sustained release was obtained up to 24 h.

Other study manufactured particles of recombinant tissue-type plasminogen activator (rt-PA) into echogenic liposomes (64), that is an FDA approved the drug for the treatment of ischemic stroke. The microfluidic device used was fabricated with PDMS and had complex flow-focusing geometry. Lipid phase was placed in the outer channel while the rt-PA was placed in the inner channel. The average size of the particles obtained was 5 µm. The encapsulation efficiency of the drug into the liposome was 69%. Despite the formation of particles, their stability was observed at least 30 min. It was found that a surfactant, Pluronic F-127, provided stability to the particles and reduced particle aggregation post-production. In this case, particle size was dependent on the lipid flow rates because the size decreased when the lipid flow increased, and drug flow was maintained constant. However, it was found that microfluidic device produced less rt-PA loaded in liposomes than conventional methods. For this reason, future studies need to be performed to solve the main drawbacks: particle stability, particle aggregation and some particles produced.

Dhar *et al.* performed an assay to treat the prostate cancer through NPs (65). NPs were assembled using a microfluidic channel to perform targeted co-delivery of cisplatin and docetaxel. The protocol was based on conjugation of PLA to succinic acid-derivatized Pt prodrug to gel cisplatin prodrug functionalized polylactide (PLA-Pt(IV)). Then, this prodrug and docetaxel were nano precipitated. Afterward, the surface was functionalized with 10-Aptameter to get targeted NPs. The NPs had a high encapsulation efficiency of hydrophilic and hydrophobic drugs. The mechanism of action of the targeted NPs consists in binding to the prostate specific membrane antigen on prostate cancer cells, and then they are internalized via endocytosis. This internalization was verified by using fluorescent dye.

Xi *et al.* developed another simple and economical method for encapsulation of two anticancer drugs using the fluidic nanoprecipitation system and PLGA polymer (66). They used different solubilities, such as paclitaxel (hydrophobic) and doxorubicin hydrochloride (hydrophilic). This system enables nanoprecipitation to encapsulate hydrophobic drugs and emulsification for hydrophilic drugs in a simultaneous way.

6. DELIVERY OF DRUGS VIA MICROFLUIDICS

The microneedles system is also a good example of drug delivery via microfluidics. Some examples of this technique include a microneedle patch

Table 3. The materials used for fabrication of drug careers in microfluidics systems

Application	Material	Ref.
Transdermal delivery of insulin	Calcium sulfate and gelatin	105
Transdermal delivery	Polyvinyl alcohol and maltose loaded with sinomenine hydrochloride	106
Rabies vaccination in dogs	Polydimethylsiloxane (PDMS)	107
Transcutaneous immunization	Polydimethylsiloxane (PDMS)	108
Polio vaccination	Polyimide-coated fused-silica capillaries	109
Insulin delivery	Polydimethylsiloxane (PDMS)	110
Donepezil hydrochloride delivery (treatment forAlzheimer´s disease)	Hydroxy-propyl-methylcellulose	111
Tetanus vaccine	Polyvinyl alcohol, sucrose, and CMC	112
Ocular drug delivery	Solid, titanium-based	113

with 57 microneedles coated with 5-aminolevulinic acid. This device showed a better and deeper penetration in skin compared to topical formulations (67). Also, electrodes were fabricated using polymeric microneedles to detect glucose and lactic acid in solution using the enzymes glucose oxidase and lactose oxidase. This biosensor showed a linear response for both glucose and lactic acid with a detection limit of up to 1 μM (68). Microneedles have recently been used for studying immunization reactions. *In vitro* analysis proved that a higher penetration with the microneedles resulted in a higher immunoglobulin G2 (igG2) titer than commercial tetanus toxoid vaccines (69).

7. MICROFLUIDICS AS A TOOL FOR DRUG DELIVERY: FROM CELLULAR TO ORGAN LEVEL

The process of drug discovery begins with the selection of the target gene or protein that can be affected by the drug, followed by optimization of the drug (either a technical or commercial approach) and clinical trials. Microfluidics technologies offer several advantages over the conventional drug manufacturing methods, such as easy manipulation of fluids with a higher precision, using minimal reagents, achieving fast reaction times. These devices can be applied for preclinical tests, dosage development, cytotoxicity studies, target selection and validation (62). The applications of microfluidics technologies in the drug-delivery area are on-site analysis, protein crystallization, compound generation, high-throughput screening, organ-on-a-chip and fabrication of drug carriers (Table 3).

7.1. On-site analysis

On-site analysis refers to the quantification of protein content, protein separation, or single amino acids analysis from a lysed cell in a microfluidic device. An examples is a device developed by Huag *et al.* that can lyse, separate and quantify the protein content in a single cell (70). Protein labeling is carried out via fluorescent-antibody binding; the molecule count is carried out with cylindrical optics. Other example is the microfluidic chip-capillary electrophoresis devices made of poly (methyl methacrylate) that allows on-site analysis by checking an ample range of biomarkers such as electrolytes or proteins with a small amount of sample (71). A heteroduplex mobility assay was developed by Chenouard *et al.* to detect mutations to differentiate between wild-type and genome-edited animals that allow easier detection and analysis of these mutations (72). Also, a carbon-polydimethylsiloxane composite electrode was developed via *in situ* polyaddition to detect the constituents in *Rhizoma chuanxiong*, a traditional Chinese herbal medicine. This device was coupled with capillary electrophoresis to separate and detect metabolites such as vanillin acid, caffeic acid and ferulic acid (73).

Particle separation based on size can be achieved via inertia, hydrodynamic effects like elasticity, among others. Viscoelasticity-induced particle separation has particularly gathered attention because of its ability to gather separation in a wide range of flow rates. This type of separation consists of pushing larger particles downstream by elasto-inertial mechanisms in a straight channel; however, the purity of the particles decreases with a bigger particle concentration (74). Liu *et al.* achieved separation of particles of a variety of sizes in a low throughput regime and tested the mechanism via cell separation in *E. coli* and red blood cells (75). A two-stage microfluidic device for sheathless particle separation was developed. Results indicated that, at the first stage the particles in a range of 5 to 10 μm diameter were concentrated at the central channel and then successfully separated at the second stage. The device was later tested for blood cell separation with positive results (76).

Evidences point to this fact that, different microfluidic systems have different hydrodynamic forces, for example, the straight channel is the simplest designs for ordering particles based on inertial forces.

In addition, multiple equilibrium positions can be used to collect different particles. Curved spiraling channels use the "dean flow," a secondary flow caused by the curvature of the channel to separate particles (77).

7.2. Protein crystallization

Protein crystallization is achieved by creating a supersaturated solution that does not disturb the natural state of the molecule. This is achieved by adding precipitating agents and modifying conditions, such as temperature or pH. The main application for protein crystallization is x-ray analysis (78). Protein crystallization is carried out via testing or screening of various chemical conditions, such as salts, buffers, and precipitating agents. Since each protein crystallizes at a very specific chemical condition, a large amount of sample is required, as well as a large amount of reagents (62). Hence different purification techniques must be applied before protein crystallization to achieve a high pure molecule such as chromatographic separation, ultrafiltration, aqueous two-phase systems, among others (79).

Microfluidic technology allows the screening of several crystallization conditions at the same time with only a small amount of protein sample. Recently, microfluidic devices have been designed to carry out crystallization trials in a nano-scale volume. A centrifuge-based microfluidic device was developed to perform crystallization tests by vapor diffusion; the device consisted of 24 chambers, two capillary stop valves, and vapor-diffusion chambers. The device was tested and compared to more conventional crystallization techniques; it was found out that this device was able to explore a wider range of crystallization conditions as well as faster reaction times and lower cost (80). Another microfluidic device was developed to test crystallization conditions via a semi-contact dispensing method. The volume of each droplet was between 4 and 8 nL. Up to 96 crystallization conditions could be analyzed in the device (81). Finally, a device capable of crystal formation at specific locations in the microchip was developed to perform statistical analysis of crystallization. The device was formed of PDMS chambers, each containing an oil/water interface, and connected to a central channel, and was able to control where in the device the crystal formation was carried out (82).

8. MICROFLUIDICS AND HIGH-THROUGHPUT SCREENING

High-throughput screening (HTS) is usually performed using multiple-well plates; micro-scale technology allows researchers to use a smaller amount of reagent and faster reaction times. Examples of these technologies are multiplexed systems, microwell arrays, plug-based systems and gradient-generating devices (62).

8.1. Multiplexed systems

This system involves the use of several valves, actuators, and pumps to control the delivery of several fluids to each chamber independently. Multiplex systems main feature is a fluidic rotatory mixer that allows multiple processes to occur at the same time. Some applications of this kind of microfluidic device include detection of food pathogens, such as *Escherichia coli* and *Salmonella* (83), screening of pesticides or drugs, such as the self-assembled biocompatible, non-toxic dual-color encoded DNAzyme nanodevice developed for screening of UO_2^{2+} and Pb^{2+} in living cells (84). Also, the immunochromatographic device based on chemiluminescence for detection of ractopamine and clenbuterol (85) and the immunochromatographic test strip developed by Shu *et al.* for pesticides residues screening using chemiluminescence and a bifunctional antibody that can recognize parathion and imidacloprid simultaneously within 22 minutes (86).

8.2. Microwell arrays

Microwell-based microfluidic devices can be applied for a range of applications, such as drug screening. A microfluidic chip that allows for drug screening in tumor cells was developed to study both the cytotoxic effect of a cancer treatment drug, such as paclitaxel or doxorubicin as well as the drug signaling pathway (87). However, cell trapping and analysis is the main application for these types of microfluidic devices. Ornoff *et al.* developed a structure comprised of microwells with hydrogel at the bottom that allowed cells to be trapped in each well (88). This structure allowed culture survival while ensuring that each cell to be separated from one another and is useful for high-throughput studies; there are also microwell systems applied to dielectrophoretic cell trapping and analysis (89) or as a measurement tool for bioleaching cultures (90). A PEG microwell system has also been developed for studying the formation of embryoid bodies (EBs) (91) and another one for studying the response patterns of calcium channels as well as quantifying single cell responses in human T-lymphocyte cells (92).

8.3. Plug-based systems

Plugs function as small containers in which reactions can be performed. This type of system can allow studies with limited volume sample, such as the study of aggregation patterns of amyloidogenic peptides in cerebrospinal fluid from mice (93). The plugs can be dispensed into another material, such as a membrane (94). Blood typing and subtyping have also been achieved with this technology: multiple agglutination assays were carried out in

Table 4. Microfluidics systems used for Organ-on-a-chip application

Microfluidic device material	Geometry	Organ/tissue	Application	Ref.
PDMS	Co-flowing	Brain tissue	Toxicological and pharmacological profiling and discovery of new drugs.	114
PDMS and glass	Complex co-flowing	Human renal proximal tubular cell line	Renal interstitial fibrosis.	115
PDMS	Complex co-flowing	Human bronchial epithelial cells	Lung cancer studies.	116
-	-	Human brain endothelial cells	Study brain barrier function.	117
PDMS	Complex co-flowing	Retinal lineage cells	Degenerative retinal diseases.	118
PMMA and PDMS	Complex co-flowing	Neonatal rate cardiomyocytes and human umbilical vein endothelial cells	Cardiovascular toxicity evaluation of pharmaceutical compounds.	119
PDMS	Co-flowing	Primary cortical neurons	Alzheimer's disease.	120
PDMS	Co-flowing	Brain tissue (axons)	Traumatic brain injuries.	121
Glass and agarose	-	Cardiomyocytes	Cardiomyocyte beating synchronization.	122
PMMA	Co-flowing (three-forked sheath structure)	Red blood cells and cardiomyocyte cells	Cell sorting.	123

parallel with the advantages of avoiding cross-contamination and using very little blood volume (95). Additionally, extraction of the plugs can be carried out magnetically, using a hydrophilic patch in a hydrophobic channel that allows the particles to be removed (96).

9. ORGAN-ON-A-CHIP

Organ-on-a-chip refers to a microfluidic cell culture device that consists of a clear polymer lined with hollow microfluidic channels that contain cells. Several of these chips can then be lined up, each of them with different types of cells, to replicate the whole body response to a drug, eliminating the need for animal testing (97). Organ-on-a-chip can overcome the disadvantages of the cell cultures phases (i.e. the difficulty in harvesting metabolites, the absence of an interphase, the lack of fluid flow). The chip is made by a technique called soft lithography, pouring a liquid polymer on a silicon substrate, generating repeating patterns in the chip. By using this technique different cells can be exposed to each other or remain independent. In addition, integrating a porous substrate to separate microchannels can be applied to monitor transcellular transport and secretion. This approach allows control over a number of parameters that other techniques such as 3-D cell cultures cannot control. The simplest organ-on-a-chip is made of a single chip containing one kind of cell to study one tissue. Systems that are more complex can be built to recreate interphases between different types of tissues and, mechanical stresses that can trigger the signaling cues can be simulated. In general, organ-on-a-chip systems allow a more precise replica of *in vivo* conditions by incorporating parameters, such as fluid shear stress, mechanical stress, and electrochemical gradients. For example, cells can be adhered to a flexible membrane to experience mechanical stress, replicating processes, such as breathing, or be exposed to an electrical field (97); cellular barrier tightness in organ-in-chips can be measured by transendothelial electrical resistance (98) and vascularization can also be emulated via fibrin scaffolding (99). By replicating the *in vivo* environment, this technology helps improve drug development and manufacturing processes, allowing testing of drug delivery systems as well as cytotoxicity assays. Table 4 presents studies related to organ on a chip.

10. MICROFLUIDICS FOR CANCER CELLS STUDIES

Microfluidic devices can be considered as the part of the first stage of cancer approach because they can be useful in the diagnosis by allowing multiplex screening of cancer cells. This is important for developing a precise, preventive and personalized way of practicing medicine. These systems can be automated and increase the efficiency of anti-cancer drug development. The single cell analysis in large scale enables the identification of heterogeneous subpopulations among the cancer cells. Several devices have been developed to screen for multiple markers simultaneously in different types of cancer. As an example, Fan *et al.* developed a device for immune-detection of 12 markers related to breast and prostate cancer, and when compared with 22 known patients the results turned out to be reliable (100). Wlodkowic *et al.* developed an assay in a device with an array of 440 traps that could immobilize leukemia cancer cells and allowed its characterization (101). This is an example of how microdevices can rise the opportunities for further studies on the development of drugs efficiency. Despite the promise of this technology, there is much

research yet to be done because the methods have not been validated with clinical samples and complex environments.

11. SUMMARY AND PERSPECTIVE

Undeniably, microfluidics is one of the remarkable achievements of the 21st century, regarding its impacts and promises in the academic studies. Microfluidics technology has already disclosed its potentials for drug delivery systems and as a platform to screen the therapeutic potential of biologically active compounds and cell-drug carrier interactions. Microfluidic systems can deliver the precise and small amount of drugs to the targets decreasing the necessity for using high concentrations of drugs with considerable side effects. Also, the usage of microfluidic technologies for multiple cells and organ cultures keeps promises for the progress and translation of novel therapies by allowing one drug to analysis the behavior and response of multiple cell lines, tissues or organs in a combined and precise controlled and well-defined system. The future direction of microfluidics as a novel and pioneering science is highly related to developments in miniaturized implantable systems for long-term therapy. Nevertheless, the progress in this area of science cannot be sustained without funding priority, innovations in the area physics and biological sciences, and a close academia-industry collaboration.

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Abbreviations: DDS: drug delivery systems, MP: microparticle, NP: nanoparticles, PDMS: polydimethylsiloxane, PMMA: poly(methyl methacrylate), PC: polycarbonate, COC: cyclic olefin copolymer, PEEK: polyetheretherketone, PIPR: polyimide plastic resin, PVA: poly(vinyl alcohol), PLA: polylactide, PLGA: poly(lactic-co-glycolic acid), PEG: poly(ethylene glycol), PCL: poly(caprolactone), SPG: shirasu porous glass, PK: pharmacokinetic, PD: pharmacodynamics, HFF: hydrodynamic flow focusing, DiI: 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate, PCL-b-PEO: poly(ε-caprolactone)-block-poly(ethylene oxide), DMF: dimethylformamide, DOXO: doxorubicin, HAS: human serum albumin, TACE: transcatheter arterial chemoembolization, PTFE: polytetrafluoroethylene, rt-PA: recombinant tissue-type plasminogen activator, igG2: immunoglobulin G2, HTS: high-throughput screening, EBs: embryoid bodies.

Key Words: Microfluidics, Drug delivery, High-throughput screening, Organ-on-a-chip, Cancer cells screening

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