Microfluidics in Drug Development from Traditional Medicine

Subjects: Others

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Microfluidic chip technology has spread throughout every step of the drug development process and plays an integral role, but classical drug development strategies are inadequate. Although numerous drugs have been produced for epidemic diseases, the mortality rate is still high globally. Over time, traditional medicine has been a powerful therapy for many illnesses. Therefore, the development of new drugs from traditional medicine is a promising strategy. New drug development from traditional medicine, either by obtaining the active ingredient directly from existing natural drugs or through direct modification of a traditional drug formulation, has received increasing attention as a powerful way to address this problem. However, there are quite a few hurdles to overcome when trying to develop new drugs from traditional medicine. Fortunately, microfluidic chip technology also plays an irreplaceable role in the strategy of new drug development from traditional medicine and can be applied to all aspects of the drug development process. However, there are currently insufficient reviews on the role of microfluidic chip technology in the development of new drugs from conventional medicine. Herein, the application of microfluidics in two major steps of drug development from traditional medicine has been described: the separation and purification of target components from complex samples and the screening of active components.

Keywords: microfluidics ; drug development ; traditional medicine ; bioMEMS

1. Separation and Purification of Target Components from Complex Samples

In response to the strategy for developing a new drug from traditional ones, whether it is to develop a drug containing a single active ingredient, to directly modify a traditional drug formulation to produce a drug, or to monitor drug concentrations in body fluids to track the body, the requirement for the isolation and purification of a single ingredient from a complex sample is inevitable. However, the extraction and purification methods widely used today are shackled. For example, Liquid–liquid extraction (LLE), as a classical sample pretreatment method, can be used for sequential extraction with different organic solvents to gradually separate the active ingredients or obtain different compounds for subsequent activity screening. However, LLE suffers from various drawbacks that limit its application, including emulsion formation, low extraction efficiency, time consumption, high organic solvent and sample use, difficulties in automation, poor exposure to toxic solvents, and discharge of waste into the environment. [1]. Therefore, new advances in extraction and purification methods are needed to facilitate the development of new drugs for traditional medicines. The advent and rapid development of microfluidic chip technology in recent decades have greatly improved the current situation. Due to the unique laminar flow properties of microfluidics, the combination with LLE can be used to achieve improved extraction efficiency, reduced sample pretreatment time, lower reagent and sample consumption, and reduced contaminant emissions. Coupled with the ease of automation, integration, and parallelization of microfluidic chips, there are additional benefits of reducing human intervention, increasing throughput, and facilitating interoperability with other equipment [2][3][4][5][6][7]. The laminar flow phenomenon of microfluidics implies that when two liquids are introduced into the main microchannel of a microfluidic chip from separate inlets, the molecules can move rapidly from one liquid to another by spreading due to the low Reynolds number. Therefore, when LLE is combined with microfluidic chip technology, it is more efficient than macro-LLE [2][5][8]. This, coupled with the ease of automation, integration, and parallelism of microfluidic chips, can reduce manual handling, increase throughput, and facilitate the coupling of other devices for additional studies. With the outstanding contribution of microfluidic technology, a qualitative leap in the field of developing new drugs has been made from traditional medicine [5][9][10]. In the next subsections, the exploration of improved facilitation of separation will be presented and purification methods by microfluidics in the process of developing new drugs from traditional medicine, including the development of drugs containing a single active ingredient from traditional medicine, quality

control of drugs from improved formulations of traditional medicine, and real-time monitoring of drug concentrations in body fluids.

1.1. Development of Drugs Containing a Single Active Ingredient from Traditional Medicine

If an attempt is made to develop a drug containing only one active ingredient from traditional medicine, as is the case with most modern drugs, then due to the complexity of traditional medicine, the action of extracting and purifying a single ingredient from a complex sample needs to be completed [2]. However, as mentioned before, traditional separation and purification methods do not support the research, and the application of microfluidic technology has a practical and effective promotion effect. In the following parts, some experimental designs will be presented on how microfluidics can be applied in separation and purification methods for the development of drugs containing only a single active ingredient.

In 2018, Rouhollah Heydarid et al. attempted to extract olive bitter glycosides from organic extracts of olive leaves into the aqueous phase using microfluidic chip technology. Olive bitter glycosides occupy an important position in medicine and can be used as antibiotics, antimalarial agents, antioxidants, and in the prevention of Alzheimer's disease. However, the current process of extracting olive bitter glycosides from resins is complex, time consuming, and expensive, making it of great importance. In this experiment, a syringe pump served as the driving force; the ethyl acetate extract and the aqueous phase entered two separate inlets; a t-shaped microchannel served as the mixing chamber; a coil was provided at the outlet to increase the contact time between the two phases; at the outlet, the two phases were separated because of the difference in density (bottom: aqueous phase and top: ethyl acetate); and the concentration of the two phases of olivetin was determined using a high-performance liquid chromatography (HPLC) system.

In a recent study, Qidan Cai et al. used the g-quadruplex approach to extract strong alkaloids from the extract of Macleaia coral seed using a three-phase laminar flow chip. This experiment allowed a free drug, a G-quadruplex reagent (HT24), and a drug bound to HT24 to enter the TPL chip. Since the free drug has a higher diffusion coefficient, it is more likely to enter the organic phase layer. In contrast, the drug bound to HT24 is left within the sample solution. Finally, the two phases are collected and detected by HPLC. Four antitumor alkaloids, pyrethroids, hesperidin, fisetin, and gallic acid, were eventually obtained by this microfluidic system [6]. Qin Weiwei et al. created two laminar flow extraction devices (three-phase flow microfluidic and continuous r flow microfluidic) on a microfluidic chip. In this experiment, the extraction efficiency of different microfluidic devices was explored by a scheme to isolate and purify ginsenosides from ginseng extract samples, and compared with the current two-phase chip. It was demonstrated that two types of laminar flow extraction can accomplish successive solvent extractions. The extraction efficiency of the successive flow chip was greater than that of the three-phase chip. In addition to the gentle extraction process based on the molecular fusion mechanism, both three-phase chips and continuous chips have the benefit of reducing complex processing and saving time [2].

Separation and purification can be improved by combining microfluidics with LLE or induced phase separation extraction (IPSE). IPSE is a modification of LLE, in which organic solvents are separated from water in seconds through the addition of salt or hydrophobic solvents or cooling of the solution to a temperature below zero. Ideally, the components can move quickly to more affinity solvents due to differences in substance properties. By using the separation principle of IPSE, a solution containing the target product can be obtained quickly. However, there is still a need for automation due to the complex operation of IPSE, and microfluidics can help reduce the complexity of the operation. For example, a valuable experiment was conducted in 2021 to develop an IPSE chip using microfluidic technology. The addition of a hydrophobic inducer resulted in the separation of the sample solution of acetonitrile–water into separate organic and aqueous phases. The adjustment in the acid/base properties of the sample solution affects the distribution of the compounds. In this experiment, aglycones and glycosides were successfully separated from *Scutellaria baicalensis* extract. Sample and solvent usage were whittled down. The sample pretreatment time was shortened, the extraction efficiency was higher than that of the LLE chip, and the formation of emulsions was prevented. More importantly, the operation is far less tedious compared to macro-LLE or IPSE [5].

In the above section, the focuses are some explorations on two-phase microfluidic chips, three-phase microfluidic chips, continuous laminar flow microfluidic chips, and IPSE microfluidic chips, all with invariably excellent results over macroscopic methods. In addition to the aforementioned microfluidic experiments for extracting the active ingredients of herbs, there are many more explorations in this area.

1.2. Quality Control of Drugs from Improved Formulations of Traditional Medicine

The drugs obtained by modifying formulations of traditional medicines require quality control to ensure the safety, efficacy, and consistency of the drug. This is because the raw material growth location, conditions, different production methods,

and differences in the drug production process may result in the drug having a different chemical composition and therapeutic effect [11]. Another reason is to prevent the addition of additives that are harmful to humans, such as formaldehyde, during the preparation process [12]. The quality control of pharmaceuticals is still mainly performed using methods that detect certain specific components in the sample, but the complexity of the sample composition may affect the detection process, leading to unreliable conclusions. Therefore, extracting the target molecule from a complex sample before performing the assay can greatly improve the accuracy of the assay results. Microfluidics can provide a very beneficial contribution to the extraction process. In the next section, some experimental designs of microfluidic techniques applied to separation and purification methods will be presented. These designs enable the quality assessment of new drugs developed by improving the formulation of traditional pharmaceuticals.

Ling Lin et al. developed a microfluidic system for the extraction and detection of catechins from green tea by combining solid-phase microextraction and chemiluminescence. Specifically, it is divided into two steps. Firstly, the monolithic column is integrated into the microfluidic chip to achieve the extraction of catechins from green tea. Then, the catechins react with potassium permanganate to produce chemiluminescence. The reason for using the monolithic column as the extraction material is its good permeability, convenience, and enrichment, while the use of microfluidics enables the automation, integration, and miniaturization of the analysis, which has tremendous benefits in terms of reduced reagent usage and time reduction. Finally, since the reaction of catechins with potassium permanganate can produce chemiluminescence directly, additional manipulation and the influence of external light sources can be avoided, and the effects caused by stray light and light source fluctuations can be excluded. In their analysis, the linear range was 5.0×10^{-9}–1.0×10^{-6} M, and the limit of detection was 1.0×10^{-9} M; the recoveries were able to reach 90%–110%; in addition, the relative standard deviation (RSD) was found to be 4.8% by parallel measurement of 10 samples with 1.0×10^{-8} M catechins. These data show that the system has satisfactory linearity, sensitivity, recovery, and precision. The system eliminated the tedious elution step and had high sensitivity, low consumption, and reusability, thus being an inexpensive and high productivity means of testing the quality of green tea [13]. Often, the therapeutic activity of conventional medicine is due to the synergistic and simultaneous action of several chemicals; therefore, confirming only the content of a single ingredient may not be realistic [14]. Since the determination of single chemical composition is not sufficient, the determination of multiple chemical compositions may be one of the favorable methods for increasing the convincing power. Attempts to control the quality of drugs by testing multiple ingredients were made more than a decade ago. A flow injection (FI)-microfluidic capillary electrophoresis (CE) microfluidic chip, suitable for the determination of the main components in pharmaceutical preparations, was proposed in 2003. This experiment determined the content of four alkaloids (ALP, SRI, MT and OMT) in two marketed drugs and proved the efficiency, reproducibility, and applicability of the FI-CE microfluidic chip. It is a promising method for both drug quality control and routine analysis and monitoring [15]. In addition, Tsung-Ting Shih et al. combined microfluidic electrophoresis and electrochemical detection with a simple derivatization method for the determination of five representative components of hesperidin, including Pinellia ternata guanosine, methionine, glycine, 3,4-dihydroxybenzaldehyde, and homogentisic acid. The improved efficiency and reduced sample and reagent consumption provided a promising idea for the efficient analysis of complex components [16]. Additionally, in 2017, Mad et al. provided an interesting experimental design for the completed biological pharmaceuticals. Ahmad et al. evaluated the quality of QiShenYiQi pills (QSYQ) using microfluorescence and assessed the biological consistency by enzyme inhibition of thrombin and angiotensin-converting enzyme (ACE) as quality biomarkers. QSYQ is a pharmaceutical product containing plants such as Astragalus, Salvia, Panax ginseng, and Dalbergia odorifera. The complexity of the constituents led to the undesirability of the strategy of quality assessment based on the presence or absence of one or several molecules within the drug. They innovated the approach of judging the effectiveness of pharmaceuticals based on the changes in the biomarkers. Three functions, namely, enzyme–MB complex formation, and enzyme reaction and screening, were combined in a multifunctional chip. Then, enzyme inhibition (percentage) was used as an indicator of drug quality; and the HPLC system combined with a variable wavelength detector (VWD) was used for a fingerprint analysis. After studying five batches of QSYQ, differences in the effects on thrombin and ACE were found between batches, demonstrating the validity of the method. More positively, they further explored the linearity, reproducibility, and reliability of this strategy. The results showed an r2 of 0.9988 and a repeatability of less than 15% for thrombin; and an r2 of 0.9810 and a repeatability of the same less than 15% for ACE. The reliability of the strategy was then demonstrated by using AEBSF-HCl and captopril as positive controls, measuring their IC50, and comparing them with literature data. Moreover, herein, not only the quality assessment was achieved, but also tthe discovery of molecules that actually have a real therapeutic effect. Tannins, B hydrochloride, tannic acid C, and rosmarinic acid showed potent inhibition of thrombin, while tannins inhibited ACE [11].

The above examples show the importance of efficacy and quality assessment of new drugs developed by improving traditional drug formulations. However, it is worth noting that the hazards in additives are also of great concern in quality control. A well-known example is “formaldehyde”. Excessive absorption of formaldehyde can lead to adverse reactions

such as headaches, abdominal pain, vomiting, and breathing difficulties [12]. Hence, ensuring that the level of formaldehyde in pharmaceuticals is within the safe range is important for human health. However, as previously shown, the separation and enrichment step is important for eliminating the influence of other compounds and accurately detecting formaldehyde in the sample. The development of microfluidics has given new ideas on how to determine the concentration of formaldehyde. Lung-Ming Fu et al. determined the concentration of formaldehyde by measuring the fluorescence intensity signal of the reactants in the collection chamber. The principle is that the samples are first mixed with a fluorescent derivatization reagent (Fluoral-P); then, heated (30 °C) for a period of time (4 min); and finally, the formaldehyde concentration is determined by the fluorescence intensity under a microscope in a laser-induced fluorescence (LIF) detection system. They used the detection system to test standard samples with known concentrations and compared the results with the traditional UV/visible absorption spectroscopy method to demonstrate the reliability of the system. The fast and easy nature of this detection system provides great convenience for the detection of formaldehyde content. In addition, by measuring the formaldehyde concentration of ten commercial Chinese medicines, the practical application value of the device was assessed [12]. The above experimental explorations are all attempts to validate that the chemical composition and therapeutic effects of the final product are influenced by differences in geographic origin, growing conditions, agricultural production methods and manufacturing processes, and the addition of additives harmful to humans during the preparation process by combining assay methods with microfluidic chip technology. These experimental studies have a very positive value and can help the use of drugs in practice, as well as provide good ideas for other scholars' research. When drugs enter the body, the real-time monitoring of changes in the concentration of certain dangerous ingredients to prevent adverse reactions is also important for human safety considerations.

1.3. Real-time Monitoring of Drug Concentrations in Body Fluids

Quantitative analysis is quite challenging in situations where a wide variety of contaminants are present in urine, saliva, plasma, and blood samples, and the target drugs are frequently present at low concentrations [3]. However, drug abuse-related adverse events are frequently reported.

To perform a rapid, convenient, and quantitative analysis of the target drug, it is necessary to minimize matrix effects, eliminate contaminants, preconcentrate analytes, and collect samples in a form that is compatible with the analytes in the instrument. So, in clinical application, the price and operation of such technologies should not be excessively high and tedious, respectively. Microfluidic technology has the benefits of low cost, high extraction efficiency, and easier implementation of automation, integration, and parallelism, allowing it to meet these needs [3]. Ephedrine (EPH) (2-methylamino-1-phenylpropan-1-OL), extracted from ephedra, is an example of a drug whose concentration must be strictly regulated. It is a central nervous system sympatholytic stimulant that prevents hypotension and stimulates adrenergic receptors, thereby increasing blood pressure and heart rate. However, misuse of the drug can result in adverse cardiovascular reactions and even death. Mahdoo Baharfar et al. created a microfluidic device that combines the benefits of electromembrane extraction and microfluidic devices, thus specifying an electromembrane extraction (EME) on a chip for extraction and preconcentration of ephedrine from human urine and plasma samples. Utilizing central composite design (CCD) technology, the effective parameters of the extraction process were optimized: low sample volume requirements, adequate sensitivity, and low limit of detection (LOD). By increasing the surface-to-volume ratio and creating a uniform electric field along the entire length of the channel, the extraction efficiency was enhanced. In addition, the small distances between the electrodes allow a low voltage to generate a large electric field. These attributes have influenced the design of portable analytical devices [17].

Jin Sheng et al. also invented a hybrid quartz capillary/polymethylmethacrylate microfluidic device (HMD) for the rapid and sensitive detection of psychotropic drugs in blood by UV light. This takes advantage of the fact that psychotropic substances can absorb UV light at 200 nm, and takes advantage of the high efficiency, reduced reagent loss, and reduced time of the microfluidic device (MED). Specifically, fused silica capillaries, which tend to form breaks at relatively low sampling voltages, can then provide separation channels and a window without cladding for these drugs. It is also worth advocating that such an assay device can be reused after electrophoresis buffer rinsing, which greatly saves costs. The results show that baseline separations for barbiturates (phenobarbital and barbiturates), benzodiazepines (nitrazepam, clonazepam, chlordiazepoxide, alprazolam, and diazepam), and a tricyclic antidepressant (amitriptyline) were achieved within 200 s with 3.8×10^5 plates m^{-1} at the maximum separation efficiency. The difference is that the conventional methods of capillary electrophoresis and high-performance liquid chromatography take 12 and 15 min, respectively. Coupled with the compact and convenient nature of the device, the design and popularity of the device are of great significance if they can meet the detection needs. Moreover, the detection limits of the device for the eight psychotropic substances were as low as 27 ng/mL (phenobarbital) and as high as 67 ng/mL (chlordiazepoxide) in plasma samples spiked with standard levels of psychotropic substances. Therefore, the device is fully capable of reaching the clinically

effective therapeutic concentration of 100 ng/mL. In contrast to the lowest concentration of 500 ng/mL that is harmful to humans, the linear range of the other seven drugs can reach the upper limit of detection of 1000.0 g/mL, except for chlordiazepoxide, which has an upper limit of detection of 2000.0 g/mL. Therefore, their sensitivity and linear range fully meet the needs of the assay. So, it provides a promising method for the preliminary screening of psychotropic drugs with high resolution, rapid separation, and low expense. Of course, it would be preferable to have a further improved strategy to be able to determine directly in the device exactly which psychotropic drug is in the sample [18].

A microfluidic device involving the use of a bio-nanopore consisting of a biological nanopore bound to a DNA aptamer has also been prepared. This chip enables on-the-spot drug detection and rapid detection of small molecules [19], even detecting cocaine at concentrations as low as 300 ng mL^{-1} in 60 s. Another microfluidic device containing a mid-infrared single-mode strip waveguide that can be used for cocaine detection has also been prepared. However, this device has a minimum detection concentration of 100 mg/mL, which is a significant shortfall compared to other assays that can detect nanogram orders of magnitude per milliliter [20].

Several recent examples of combining microfluidic chip technology with monitoring methods have been highlighted to demonstrate the superiority and universality of microfluidic chip systems. It is believed that this can provide better ideas for more experimental design solutions, and more applications of microfluidic chip technology are looking forward.

2. Screening of Traditional Medicine Active Ingredients

Regardless of the approaches to drug development from traditional medicine, drug screening is a mandatory part of the process. However, traditional in vitro cellular models and in vivo animal models are not suitable options. Based on cell colorimetric assays, microtiter plate-based cytotoxicity assays, such as MTT bromide (3-[dimethylthiazol-2-yl]-2,5-diphenyltetrazolium) assay, are widely used as in vitro cellular models for drug screening. However, the conventional test is time-consuming and involves many herbal components. In addition, since the test uses a colorimetric method, antioxidants and colorants may alter their reliability and sensitivity. This, coupled with the inability to replicate changes in the in vivo microenvironment, has led to unpredictable changes induced by drugs in vivo. In vitro animal models likewise have a non-negligible impact on the drug screening process. The high cost of animal models, the difficulty in handling them, the difficulty of genetic and physiological differences in humans, and the inevitable ethical issues pose obstacles to drug development [21]. In addition to the problems mentioned above, because the therapeutic activity of conventional medicine is often due to the synergistic and combined interaction of multiple molecules, obtaining truly valuable findings is non-negotiable; this often requires the testing of large chemical combinations and relies on data at multiple qualitative and quantitative levels [22]. These factors have shown the need to look for new drug screening methods to advance the drug development enterprise. Microfluidic platforms are an attractive option for high-throughput analysis during drug development. This is because a multifunctional integrated system based on microfluidic chips offers low cost, high throughput, speed, sensitivity, specificity, and affordability. In addition, it has the great advantage of mimicking the human environment. Therefore, the application of microfluidic chip technology can effectively solve the problems of complexity and ambiguity of the mechanism of action of traditional medicines and advance the process of drug development [23][24]. In this section, classical examples are given to introduce improved variations in experimental design in this area, with the expectation of providing good ideas for drug development from traditional medicine.

Microfluidic chips that detect pharmacological effects by measuring changes in Ca^{2+} were introduced in 2008. Ca^{2+} elevation is a well-known early cytotoxic event. The use of v-shaped microfluidic chips to pick single cells not only reduces the cost of reagents and the requirement for cells but also discloses several phenomena that are not visible in the bulk analysis due to cell heterogeneity. More importantly, activities that would have taken days in a traditional experiment were completed in hours, with comparable outcomes.

In 2017, two teams employed microfluidic technology to uncover active components in natural substances and investigate their mechanism of action to combat lung cancer. Even though FAN Jia-xin et al. concluded that Nepeta must have a certain inhibitory impact on lung cancer cells based on a past literature study and pharmacodynamic trials, the pharmacological mechanism remains unclear. They used a microvalve-structured double-layer composite chip combined with ultra-high performance liquid chromatography–mass spectrometry to analyze the ethanol extract of Nepeta mustard based on the chromatographic retention time, molecular fragmentation peak, accurate molecular weight, database, and control. Comparing the product information and analyzing and identifying the chemical makeup, five components play a significant role in suppressing the proliferation of lung cancer cells, as demonstrated by the results of the experiments [25]. However, herein it did not explore the pharmacological and pharmacodynamic aspects in sufficient depth. In contrast, a study by Jiaxin Fan et al., targeting flavonoids (TFS), provided valuable insights. In their investigation, they utilized a microfluidic chip combined with flow cytometry to examine the effect of TFS isolated from Nepeta mustard on the human

lung cancer cell line A549. To investigate the molecular mechanism of a portion of the PI3K-AKT pathway, quantitative real-time PCR (qRT-PCR) and Western blot were employed to assess the mRNA and protein expression of microRNA-126 (miR-126), VEGF, PI3K, and PTEN. The findings indicated that TFS could inhibit the expression of miR-126, modulate the PI3K-AKT signaling pathway, and exert anticancer properties [26].

There is a clear example of the close relationship between energy metabolism and inflammation in activated microglia. In addition, *Rhodiola* contains sorbide (Sal), which inhibits microglial hypoxia-induced inflammation (HI). However, it is unknown if sorbide (Sal) inhibits microglial hypoxia-induced inflammation (HI) by altering microglial energy metabolism. A new cell microfluidic chip mass spectrometry (CM-MS) system has enabled real-time monitoring of the effects of drug metabolites on cells to investigate the changes in metabolic processes during hypoxia [27]. In the meantime, they measured the level of HI-related factors to confirm the metabolic mechanism of hypoxic BV2 cells. The correlation between DFO-induced BV2 cell HI and energy metabolism was revealed by the integration of detected results. In addition, the administration of Sal could effectively reverse this transformation, and two Sal metabolites were identified: tyrosol and 4-hydroxyphenylacetic acid. This experiment reveals the mechanism by which Sal mediates energy metabolism mechanisms to reduce HI injury in BV2 microglia by promoting glycolysis to OXPHOS under hypoxic conditions. It paves the way for real-time, online, dynamic monitoring of the energy metabolism mechanism of drug effects on cells and provides a superior screening strategy for natural drug candidates for the treatment of brain diseases [27]. NijiaWang et al. designed an experiment to investigate the mechanism for TTS therapy in cervical cancer. In this experimental design, numerous techniques were combined, such as 3D microfusion, microfluidic chip, flow cytometry, mass spectrometry coupling, and bioinformatics. The results demonstrate that TTS first stops the transformation of cells in the G0/G1 stage to the S and G2/M stages. Subsequently, gene and protein synthesis was inhibited. Finally, it reached the goal of blocking cell proliferation and inducing apoptosis. Consequently, the experiment confirmed the effect of tannin on the associated genes and proteins [28].

References

1. Tetala, K.; Swarts, J.; Chen, B.; Janssen, A.; van Beek, T. A three-phase microfluidic chip for rapid sample clean-up of alkaloids from plant extracts. Lab A Chip 2009, 9, 2085.
2. Qin, W.; He, Y.; Xiao, J.; Liang, S.; Wang, S.; Li, P.C.H.; Sun, Y. A successive laminar flow extraction for plant medicine preparation by microfluidic chip. Microfluid. Nanofluidics 2019, 23, 61.
3. Baharfar, M.; Yamini, Y.; Seidi, S.; Karami, M. Quantitative analysis of clonidine and ephedrine by a microfluidic system: On-chip electromembrane extraction followed by high performance liquid chromatography. J. Chromatogr. B 2017, 1068–1069, 313–321.
4. Mu, X.; Liang, Q.; Hu, P.; Ren, K.; Wang, Y.; Luo, G. Selectively modified microfluidic chip for solvent extraction of Radix Salvia Miltiorrhiza using three-phase laminar flow to provide double liquid–liquid interface area. Microfluid. Nanofluidics 2009, 9, 365–373.
5. Shen, Y.; Chen, B.; Zuilhof, H.; van Beek, T. Microfluidic Chip-Based Induced Phase Separation Extraction as a Fast and Efficient Miniaturized Sample Preparation Method. Molecules 2020, 26, 38.
6. Cai, Q.; Meng, J.; Ge, Y.; Gao, Y.; Zeng, Y.; Li, H.; Sun, Y. Fishing antitumor ingredients by G-quadruplex affinity from herbal extract on a three-phase-laminar-flow microfluidic chip. Talanta 2020, 220, 121368.
7. Gao, H.; Yan, C.; Wu, W.; Li, J. Application of Microfluidic Chip Technology in Food Safety Sensing. Sensors 2020, 20, 1792.
8. Tang, Y.; Soroush, F.; Sheffield, J.; Wang, B.; Prabhakarpandian, B.; Kiani, M. A Biomimetic Microfluidic Tumor Microenvironment Platform Mimicking the EPR Effect for Rapid Screening of Drug Delivery Systems. Sci. Rep. 2017, 7, 9359.
9. Liu, Y.; Huang, R.; Xiao, B.; Yang, J. The Microfluidic-chip Technology and the Applications in thenScreening on the Active Comonents of Natural Product. Nat. Prod. Res. Dev. 2010, 7, 9359.
10. Amatatongchai, M.; Hofmann, O.; Nacapricha, D.; Chailapakul, O.; deMello, A. A microfluidic system for evaluation of antioxidant capacity based on a peroxyoxalate chemiluminescence assay. Anal. Bioanal. Chem. 2006, 387, 277–285.
11. Li, Z.; Ai, N.; Yu, L.; Qian, Z.; Cheng, Y. A multiple biomarker assay for quality assessment of botanical drugs using a versatile microfluidic chip. Sci. Rep. 2017, 7, 12243.
12. Fu, L.; Wang, Y.; Liu, C. An integrated microfluidic chip for formaldehyde analysis in Chinese herbs. Chem. Eng. J. 2014, 244, 422–428.

13. Lin, L.; Chen, H.; Wei, H.; Wang, F.; Lin, J. On-chip sample pretreatment using a porous polymer monolithic column for solid-phase microextraction and chemiluminescence determination of catechins in green tea. Anal. 2011, 136, 4260.
14. Thomford, N.; Senthebane, D.; Rowe, A.; Munro, D.; Seele, P.; Maroyi, A.; Dzobo, K. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. Int. J. Mol. Sci. 2018, 19, 1578.
15. Cheng, Y.; Chen, H.; Li, Y.; Chen, X.; Hu, Z. Separation and determination of aloperine, sophoridine, matrine and oxymatrine by combination of flow injection with microfluidic capillary electrophoresis. Talanta 2004, 63, 491–496.
16. Shih, T.; Lee, H.; Chen, S.; Kang, C.; Shen, R.; Su, Y. Rapid analysis of traditional Chinese medicinePinellia ternataby microchip electrophoresis with electrochemical detection. J. Sep. Sci. 2017, 41, 740–746.
17. Poe, S.; Cummings, M.; Haaf, M.; McQuade, D. Solving the Clogging Problem: Precipitate-Forming Reactions in Flow. Angew. Chem. Int. Ed. 2006, 45, 1544–1548.
18. Sheng, J.; Lei, J.; Ju, H.; Song, C.; Zhang, D. Rapid ultraviolet monitoring of multiple psychotropic drugs with a renewable microfluidic device. Anal. Chim. Acta 2010, 679, 1–6.
19. Campos, K.; Coleman, P.; Alvarez, J.; Dreher, S.; Garbaccio, R.; Terrett, N.; Tillyer, R.D.; Truppo, M.D.; Parmee, E.R. The importance of synthetic chemistry in the pharmaceutical industry. Science 2019, 363, eaat0805.
20. Chang, Y.; Wägli, P.; Paeder, V.; Homsy, A.; Hvozdara, L.; van der Wal, P.; Di Francesco, J.; de Rooij, N.F.; Herzig, H.P. Cocaine detection by a mid-infrared waveguide integrated with a microfluidic chip. Lab A Chip 2012, 12, 3020.
21. Li, X.; Xue, X.; Li, P. Real-time detection of the early event of cytotoxicity of herbal ingredients on single leukemia cells studied in a microfluidic biochip. Integr. Biol. 2008, 1, 90–98.
22. Neužil, P.; Giselbrecht, S.; Länge, K.; Huang, T.; Manz, A. Revisiting lab-on-a-chip technology for drug discovery. Nat. Rev. Drug Discov. 2012, 11, 620–632.
23. Wang, H.; Li, T.; Bao, Y.; Wang, S.; Meng, X. A multifunctional integrated simultaneously online screening microfluidic biochip for the examination of “efficacy-toxicity” and compatibility of medicine. Chin. Chem. Lett. 2019, 30, 403–405.
24. Valente, K.; Khetani, S.; Kolahchi, A.; Sanati-Nezhad, A.; Suleman, A.; Akbari, M. Microfluidic technologies for anticancer drug studies. Drug Discov. Today 2017, 22, 1654–1670.
25. Schuster, B.; Junkin, M.; Kashaf, S.; Romero-Calvo, I.; Kirby, K.; Matthews, J.; Weber, C.; Rzhetsky, A.; White, K.P.; Tay, S. Automated microfluidic platform for dynamic and combinatorial drug screening of tumor organoids. Nat. Commun. 2020, 11, 5271.
26. Fan, J.; Bao, Y.; Meng, X.; Wang, S.; Li, T.; Chang, X.; Yang, G.; Bo, T. Mechanism of modulation through PI3K-AKT pathway about Nepeta cataria L.’s extract in non-small cell lung cancer. Oncotarget 2017, 8, 31395–31405.
27. Fan, F.; Xu, N.; Sun, Y.; Li, X.; Gao, X.; Yi, X.; Zhang, Y.; Meng, X.; Lin, J. Uncovering the Metabolic Mechanism of Salidroside Alleviating Microglial Hypoxia Inflammation Based on Microfluidic Chip-Mass Spectrometry. J. Proteome Res. 2021, 21, 921–929.
28. Wang, N.; Wang, J.; Meng, X.; Bao, Y.; Wang, S.; Li, T. 3D microfluidic in vitro model and bioinformatics integration to study the effects of Spatholobi Caulis tannin in cervical cancer. Sci. Rep. 2018, 8, 12285.