Engineering of MEMS-based microfluidic devices for individualised biomedical applications

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Engineering of MEMS-Based Microfluidic Devices for Individualised Biomedical Applications

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ABSTRACT

Individualised medicine, which aims to comprehensively optimise diagnostic and therapeutic strategies for individual patients, has been greatly advanced by the recent development of novel devices based on micro-electro-mechanical system (MEMS) technology. This dissertation presents two approaches that make use of microfluidics & MEMS technology for design, fabricate and test lab-on-a-chip devices intended to provide individualised medicine. The first approach is an implantable drug delivery device for localized administration of chemotherapy. The device enables the efficient and controllable delivery of chemotherapeutic drugs to tumors to enable the reduction of side effects during treatment. The device was tested using cancer cells and animal models to evaluate its reliability and safety for clinical use. In the second approach, we developed two prototypes of lab-on-a-chip platform for cancer drug screening and testing. Its low cost, ease of use and flexibility were designed to meet the requirements of individualised medicine. Using this platform, we demonstrated drug testing on cultured cells and multicellular spheroids to investigate novel cancer drugs.

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CHAPTER 1 INTRODUCTION

This dissertation presents two approaches that make use of micro-electro-mechanical system (MEMS) technology in the design, moulding, fabrication and testing of microfluidics biomedical devices in an attempt to implement individualised medicine. The first approach provides efficient and controllable drug delivery to disease sites by using an implantable pump device in each patient. The second approach focuses on the development of lab-on-a-chip platforms for cancer drug screening and testing on cells and tissues, with the benefits of low cost, ease of use and flexibility, to deliver individualised treatment. In this introduction, we discuss the concept of individualised medicine and present background information about the use of MEMS-based devices in individualised biomedical applications.

1.1 Individualised Medicine

In recent years, individualised diagnostic and therapeutic techniques for the treatment of many diseases have attracted attention for their potential in several evolutionary biomedical researches and clinical approaches [10-12]. The concept of individualised medicine, which is also called personalised medicine in a few studies, was created for the purpose of tailoring the best treatment for each patient. In conventional medicine, patients are diagnosed with a certain type of disease by means of settled diagnostic protocols and are then treated with standardised prescriptions and plans usually with little concern for the individual patients' responses to the treatment. There are quite a few serious disadvantages of generalised medicine have recently been outlined [12]. Firstly, the generalised treatment does not allow for flexibility in the treatment of complicated diseases, such as cancer, in which complex drug formulations and real-time treatment strategies must be determined to maximise the treatment effects and minimise the side effects for each patient [9, 13, 14].

Secondly, the accuracy of the diagnosis and monitoring of a disease is crucial in conventional medicine, but it is strongly dependent upon testing instruments and technical manpower. However, all patients who have the same risk of disease cannot share the same quality of medical resources [15-21], which makes medical care in areas with limited resources difficult.

The goal of individualised medicine is to comprehensively optimise diagnostic and therapeutic strategies to treat each patient at the most precise and effective level. Differences amongst patients are highly valued in individualised medicine to help designing unique treatment plans that can best meet each patient's needs [12]. Individualised medicine also concerns the use of diagnostic and therapeutic techniques to cure patients in any setting and at any time [12]. However, it is obvious that great challenges still exist in realising individualised medicine. For example, providing medical devices for each patient's needs would require highly flexible and customisable designs to accommodate different cases with large variations. Moreover, the application of medical devices in limited-resource conditions would be challenging without the assistances from hospital or laboratory instruments and manpower. Novel biomedical technologies must be developed to achieve individualised diagnosis and treatment.

1.2 MEMS-Based Microfluidics Biodevices for Individualised Medicine

The hopes of realising individualised medicine can be seen in the recent development of MEMS-based microfluidics biomedical devices. Microfluidics is a newly emerging technology that allows the manipulation of solutions in small volumes (1 to 100 micrometers); it originated in the 1990s in the semiconductor industry [20]. More specifically, the fabrication techniques for microfluidics devices are derived from MEMS technology, in which devices are manufactured in terms of micrometers and nanometers [22]. These miniaturised applications greatly enhance the capability of handling small objects in solution

such as bacteria, viruses, cells and nanoparticles, and thus advanced applications such as targeted delivery [23], single-cell testing [4] and gene delivery [24] have for the first time become possible for researchers. Other advantages of microfluidics devices include great accuracy, high throughput, low power consumption, and a reasonable cost. With the benefit of microfluidics, a number of new biomedical applications have been developed, such as lab-on-a-chip testing, human organ *in vitro* simulation and implanted diagnosis/treatment. From the perspective of medical products, microfluidic biodevices possess several unique features, including user friendliness, ease of design and fabrication, long-term reliability, great flexibility and great portability [18, 25]. These advantages have prompted microfluidic tools for futuristic individualised medicine, which raises the demands for next-generation medical devices to deal with individual patients' needs.

The objective of this thesis is to develop devices that are based on the MEMS-based microfluidics technology and the concept of individualised medicine, at the same time, to study the potentials of using such devices in realizing individualised cancer treatment. **Figure 1.1** demonstrates the concept of using microfluidic diagnostic and therapeutic devices into the development of next generation drug discovery, drug delivery and treatment analysis. In this dissertation, we report on two microfluidic approaches towards individualised medicine that we developed. For the first approach, we engineered a MEMS implantable drug delivery device for localized administration of chemotherapy that capably defined an implanted treatment for highly efficient drug delivery and controlled treatment to allow special plans to be processed on an individual basis. For the second approach, microfluidics drug testing platforms were introduced. Biological models from single-cell, monolayer cells and tissue-linked three-dimensional (3D) multicellular models were cultured on microfluidic lab-on-a-chip devices and followed with cancer drug screening and testing applications.

Figure 1.1: Illustration of the concept of using microfluidic device in developing next generation medicine. **a.** In conventional medicine, a certain drug treatment to patient is after a long term and high cost steps from the discovery of the drug, in vitro cell study, in vivo animal study, clinical trial and drug administration's approve. **b.** Microfluidic devices show their ability to perform cell-on-a-chip and human-on-a-chip testing, which are seen as the substitutions of traditional cell and animal testing (discuss in Chapter 4). Successful drugs can be treated to patient by a drug delivery device to achieve precision, controllability and efficiency in the drug treatment (discuss in Chapter 3). Owing to the low cost and convenience in using microfluidic device, analysis and treatment can be individually tailored for a specific patient, thus the individualised medicine can be greatly promoted.

1.3 Overview of the Chapters

In Chapter 1, we present an introduction to the emerging field of individualised medicine and MEMS-based microfluidic biodevices that advance the development of individualised medicine.

Chapter 2 reviews two focused approaches to implement MEMS-based microfluidic devices. Each approach is then introduced with its aims, current development, technical features and existing challenges (Section 2.1 and 2.2). Then the state-of-the-art design and fabrication technologies for both individualised microfluidic biodevices are summarised (Section 2.3).

Chapter 3 presents the design, moulding, fabrication and testing of a MEMS implantable drug-delivery device for localized administration of chemotherapy. The developed device enables programmable chemotherapy to achieve individualised treatment effects and is demonstrated in *in vitro* cancer colony inhibition experiments (Section 3.2). Furthermore, the developed devices were implanted into *in vivo* Kunming mice and tested to evaluate their biocompatibility and working performance under implantation conditions (Section 3.3).

In Chapter 4, we discuss the prototype microfluidics drug testing devices used to test cancer drugs in biological models. The design, modelling, fabrication and benchtop experiments of the two platforms are discussed. For the first platform, a cell-on-a-chip device is introduced (Section 4.2). This platform integrates actuators, controllers and fluid networks to automatically maintain an appropriate microenvironment for culturing cancer cells and conducting tests on cells. We applied this platform in the treatment of pancreatic cancer and successfully determined a suitable formulation to overcome drug resistances within a pancreatic cancer cell line (Panc-1). Next, the second generation of microfluidics culture

device was developed. This device enables the culturing of 3D multicellular tumor models on microfluidics devices for the study of comprehensive tumor drug delivery (Section 4.3). The tumor-on-a-chip device was incorporated with an implantable drug delivery pump for pre-evaluation of tumor inhibition under different drug infusion patterns.

Finally, Chapter 5 gives the conclusions of this dissertation.

CHAPTER 2 LITERATURE REVIEW

2.1 Implantable Drug Delivery Devices for Individualised Treatment

In order to get ideal effects, individualised treatment requires carefully tailored drug administration such as timing and amount of each dosage [26, 27]. However, in conventional drug delivery methods, which include oral drug pills, topical drug gels, nasal drugs, pulmonary aerosolized medication and drug injections subcutaneously or intravenously, a great challenge is still existing [28]. For example, a higher amount of drug than the theoretically demand (overdose) is usually taken into a prescription when the treatment is performed by means of oral and topical drugs in most diseases. The reason is due to the low drug delivery efficiency that only allows a relatively small fraction of the drug to be transferred to the diseased areas. To compensate the penetration and absorption in various physiological barriers, a higher dosage has to be performed to eventually gain sufficient therapeutic effects for curing the diseases [29, 30]. As a result of overdose, serious side effects would occur. For the drug injections subcutaneously or intravenously, they can only be performed within tissues accessible by syringe needles. Also, patients will experience the sensation of physiological trauma under a frequent injection of drugs, such as in the treatment of asthma and diabetes [27]. Moreover, regarding the precise control of drug release into tissues, which is strongly demanded by individualised medicine, conventional drug delivery methods cannot address this concern [31]. This challenge becomes more serious in cancer treatment, where the anti-cancer agents are usually toxic to other normal organs than tumors thereby a precise drug delivery is highly required [32].

To overcome such limitations of conventional treatments, recently researchers have proposed the use of implantable MEMS drug delivery devices [33, 34] for controllable and

flexible targeted therapy in many diseases [7, 26, 28-30, 32, 35-50]. With the benefit of the development of MEMS technology, implantable drug delivery devices are able to be highly miniaturized. Therefore the implantation in the close proximity or directly to the disease sites becomes possible. This technique is named localized treatment (**Figure 2.1**). Successful localized treatment breaks the difficulties for the drug molecules to penetrate physiological barriers [29] thereby guaranteeing the delivery efficacy and precision, while at the same time minimizing the side effects by delivering strictly the proper amount of drug [30, 48]. Generally speaking, a well-modified device using MEMS technology enables precise loading of the drug dosages, flexible delivery of treatment profiles, and localized targeting the drugs to the disease sites; therefore individualised treatment can be carefully performed by reflecting the needs of each patient [12, 48]. In this section, the state-of-the-art approaches of implantable drug delivery device are reviewed.

Figure 2.1: Illustration of cancer treatment with an implantable device: Implantable drug delivery device can be placed closely to tumor. Then localized treatment can be performed [9].

2.1.1 Implantable Drug Delivery Microchips

Currently, one type of implantable drug delivery devices gains increased acceptance and usage. These devices are made on a thin substrate thus it has a chip-like structure and drug delivery capacity by micro-fabricating an array of micro-reservoirs (10 – 200 nL capacity of each reservoir), micro-channels and micro-valves through etching and material adding in this substrate. Several approaches combine micro-needles on the device for drug delivery locally. The micro-reservoirs are used for storing and diffusing drug in micro-volumes through selectively opening each micro-reservoir [49]. Surface micromachining and etching techniques are mostly being used in the fabrication of these drug delivery microchips (**Figure 2.2**). Several works have been demonstrated showing their great potential for various in vitro [38, 40, 51, 52], in vivo [43, 46] and even clinical applications [7].

Selectively opening of the micro-reservoirs serves as the key factor in regulating the designed drug diffusive patterns into the diseased tissues. This can be achieved by using various methods, such as membrane dissolution, sensitive hydrogels/polymers displacement, magnetically triggered particles movement, and electrothermal melting [41, 52-54]. Membrane dissolution is one of the most commonly used mechanisms for designing micro-reservoir's opening. For example, in Santini et al.'s design, a gold membrane is patterned to seal each reservoir. Then membrane dissolution was initiated by applying a potential difference across the gold membrane, thus the electrochemical dissolution of gold material would eventually open up the reservoir and release the drug to the local environment [53]. Low power consumption within a few Watt and a rapid response time within 1 to 2 minutes were listed as the advantages of the device [47]. In other devices, biodegradable polymer materials have been used for the dissolvable membranes [41, 55, 56]. For a commonly used example, poly-(lactic-co-glycolic acid) (PLGA) degrades after implantation as the result of hydrolysis in the presence of bio-fluids, which is studied in microchips.

For other approaches than dissolved membranes, hydrogel based release methods recently attracted attentions. Engineered hydrogel rapidly swells or de-swells responding to changes in the surrounding environment, such as pH, glucose concentration, temperature, and light intensity[38]. Therefore, microchips constructed by hydrogel enjoy a unique self-controlled drug release feature as the delivery would dynamically react to the changes in the surrounding environment. For example, inflamed tissues have a different pH than normal ones [38]. The triggers can also be induced manually by introducing other agents through drug intakes or injections. It is worth to mention that hydrogel's transformation is a passive feature, hence simplifies the drug delivery system by eliminating the needs for power components. Last but not least, hydrogels are highly biocompatible thus ensuring the safety of the device implantation.

However, the major disadvantage of drug delivery microchip is that there are no available re-filling methods for these devices' drug reservoir. Therefore, such devices cannot

Figure 2.2: The first in-human tested drug delivery microchip: **a.** Device photograph. **b.** Layout of micro-reservoirs. **c.** Illustration of drug diffusion after reservoir opening [7].

(Image reproduced with permission from American Association for the Advancement of Science)

be used in applications with long term treatments because the replacement of the whole device through a surgery would be necessary if the treatment must be continued [57]. Furthermore, the importance of drug refilling also includes the flexibility in modifying device's delivery contents during the treatment period. With refilling techniques, doctors would be able adjust on the prescriptions according to the patient's responses. For example, cancer could build up strong resistance to the drugs [58].

2.1.2 Implantable Drug Delivery Micropumps

In order to address the previously mentioned challenges for the long term drug treatments, a new type of implantable drug delivery device has been developed. This design utilizes a micro-fabricated fluidic pump for driving the drug solutions out of a single reservoir [59]. Drug delivery to certain tissues is achieved through a long cannula attached on the outlet of device's reservoirs. Therefore drug flows can be precisely guided to disease areas (**Figure 2.3**). The implantable drug delivery micropump is designed to be easily refilled by using a syringe which can re-inject liquid into the relatively large reservoir. This method has been demonstrated in subcutaneously implanted devices [60-62]. In comparison with multiple reservoirs microchips, the single reservoir with a micropump offers many unique advantages, such as the controllability of delivery flow rates, the ease and flexibility for both refilling of device and the implantation processes. To date, these devices have been studied for *in vitro* [6, 29, 32, 37, 62], *ex-vivo* [30] and *in vivo* [63, 64] applications, and show great potential to be further improved with additional features for precision advanced diagnosis and treatment.

A micro-fabricated micropump is the core component in such a drug delivery device and determines its therapeutic performance. Micropumps can be classified into two major categories : (i) Mechanical, which includes electrostatic, electrochemical, piezoelectric and thermopneumatic pumps, as well as those using bimetallic actuator that generates

Figure 2.3: Photography of drug delivery micropumps [6]. (Image reproduced, with permission from IEEE)

displacement for pumping, and (ii) Non-mechanical pumps like electro-osmotic, electrowetting, and capillary pumps that rely on surface tension forces [26, 27]. Mechanical pumps are generally preferred in implantable drug delivery devices due to their high actuation efficiency, excellent controllability, and low power consumption. Among the mechanical designs, electrochemical actuators have attracted attention [29, 30]. An electrochemical actuator is simple in design, as it consists only of a pair of metal electrodes. By supplying bias voltages onto the electrode, water electrolysis reaction would be initiated that generate gases that provide driven forces on drug solutions (e.g. via a membrane)[30]. Further development of electrochemical actuation focused on reducing the electro-oxidization of drugs loaded in the reservoir, which may eliminate the effectiveness of the drug formulation. Li et al. introduced a bellow to separate the electrolyte (DI water) and the drug solution loaded [42, 44, 65]. Gases were enclosed inside the bellow while at the same time pneumatic forces deflect the bellows to drive drug solutions [29, 44, 65]. A wide range of delivery flow rates (1~30 μ l/s), fast responses (< 1 second) and small of pump's size are the major advantages of electrochemical micropumps.

For another commercialized mechanical micropump, IPRECIO, is an implantable infusion pump for *in vivo* applications. It is equipped with an integrated electromagnetic

rotation micro-pump and it was tested with several implanted drug treatment studies [62, 66]. The device was powered by an integrated battery and controlled by circuits, which offers communication wirelessly with an external transmitter. Drug infusion flow rates from 0.2 μ l/s to 30 μ l/s can be achieved.

2.2 Microfluidic Culture Devices: Cell-on-a-Chip and Organ-on-a-Chip in Individualised Medicine

Individualised medicine proposes customized prescription focusing on each patient's needs thus it would rely on a more concise process to verify a drug's effectiveness. Conventional drugs target curing only certain type of diseases. Nowadays' protocols for drug testing include *in vitro*, *in vivo*, *ex vivo*, pre-clinical, phase 1, 2 and 3 clinical trials studies, and they must be conducted before a new drug is approved by administration (e.g. FDA) then the drug can be eventually launched into clinical application. This process usually means decades of experiments and requiring millions of dollar. Nonetheless, current protocols are not suitable for futuristic individualised drug testing, in which the process must be completed during a constrained treatment window of one patient. Novel drug test protocols shall be proposed when facing the needs from individualised medicine.

Microfluidic technologies offer great advances for individualised drug discoveries and drug testing. Recent approaches originated from microfluidics and lab-on-a-chip technologies showed a number of fast, inexpensive and comprehensive drug testing studies which use microfluidic culture devices. By properly defining a microfluidic environment on-chip, cell culturing parameters including medium infusion, proper temperature, gases supplies and adhesive surfaces can be maintained inside the cell culturing chambers while at the same time they provide the capabilities to easily and accurately manipulate tested drug solutions in micro volumes to any desired cell culture chamber. To date, microfluidic culture devices have shown their potential to substitute conventional drug testing interfaces. In this section, such techniques for microfluidic cell culturing are demonstrated. Further, based on cell culturing, single cell testing and organ-on-a-chip microfluidic technologies are reviewed.

2.2.1 Microfluidics Culture Devices

Cell culturing in a Petri dish was developed a century ago, and it is still the most widely-used method in most biomedical studies nowadays [67]. Novel approaches for cell culturing in numerous miniaturized culture chamber on-a-chip are developed as well. A suitable environment is created and maintained inside each cell culturing chamber by controlling the mediums, buffers and other culturing agents. Moreover, biological parameters including temperature [68], humidity, concentration of gases (e.g. CO₂[69], O₂[70]) are needed to be carefully maintained. Using incubator is a straightforward method but it relies on a bulky off-chip instrument. To overcome this challenge, stand-alone culture devices were proposed recently. For example, oxygen concentrations gradient is a key factor for many biological responses. Maharbiz et al. have demonstrated an on chip cell culturing applications with electrochemical oxygen generator for gas supplies [70]. Specifically, power induced on patterned Ti/Pt electrodes would initiate water electrolysis and generate oxygen gases. Temperature is another crucial factor in biomedical tests, especially a stable 37.5 °C is necessary human *in vivo* environments. In order to achieve on chip temperature controlling, Lin et al. have fabricated on-chip micro heaters that made use of indium tin oxide (ITO) to maintain the temperature for cell culturing [68].

The surface properties of substrate also greatly affect cell culturing. Investigations showed that surface wettability [71, 72] and roughness [72, 73] impact the adhesion, movement and proliferation of cells in culturing devices. The majority of microfluidics devices are realized using PDMS, but the hydrophobic PDMS surface is not suitable for cell adhesion [74]. Therefore, methods such as plasma treatment, UV treatment, coating of hydrophilic layers, and microstructure patterning have been developed to optimise the PDMS surface wettability [71, 74-77]. It has been demonstrated that plasma treatments on the PDMS surface increased wettability and resulted in better cell adhesion [71, 75]. By coating perfluorosulphonic acid (Nafion®) and polytetrafluoroethylene (Teflon®) on the substrate

surface, the improvement of cell adhesion has been reported [78]. Alternatively, microtextures patterned on device surfaces were proved to enhance cell adhesions and proliferation during culturing [72].

2.2.2 Single Cell Manipulation and Organ-on-a-Chip

Microfluidics culture devices introduced novel single cell trapping/culturing techniques and 3D cell-to-tissue culturing techniques. The ability of culturing complex biological models promoted microfluidic culturing devices into powerful tools for biomedical researches. Specifically patterned micro-structures or micro-channels sizes comparable to that of a cell are able to capture a single cell [79, 80]. For example, Carlo et al. have demonstrated a single cell culture array by patterning PDMS U-shaped microstructure on glass for trapping and culturing cancer cells (**Figure 2.4**) [4]. Besides, optical tweezer [81] and surface acoustic waves [82] have been demonstrated together with microfluidics devices for single cell manipulation and culturing. In cancer diagnosis, isolating and study the rare tumor cells from patient's sample (e.g. circulating tumor cells in blood) gives the opportunity to monitor cancer. The circulating tumor cells provides a comprehensive yet non-invasive means for characterizing tumor molecular subtypes, which can be utilized for stratifying patients to appropriate cancer therapy. For example, Khoo et al. reported that the proliferation of circulating tumor cells is correlated with patient's survival by culturing them after being harvesting from the patients [83]. In cancer treatment, drug assay on single cells can be easily performed with lab-on-a-chip, which can provide information for evaluating treatment effects. Besides cancer, single cell manipulation is also useful in studying neuron cells. For example, Robinson et al. fabricated an array of vertical nanowires on-chip to isolate single neurons, and these nanowires also served as electrodes which intracellularly record and stimulate neuronal activities [84].

Figure 2.4: Microscope image of U-shaped microstructure trapping a single cell [4].

(Image reproduced with permission from Royal Society of Chemistry)

Aside from single cell culturing, multiple cells' group cultured in three dimensions into living organs have attracted attention in recent years [85]. Conventional mono-layer cell culturing techniques have been applied as the most fundamental biological models in cell biology studies. However, a mono-layer cell culture is too simple to mimic the *in vivo* tissues for drug testing applications [86]. Factors like mechanical forces, cell to cell signalling and nutrients/drugs gradients are hardly achieved by monolayer cell culturing but they are crucial factors in pathophysiology [85, 87, 88]. Microfluidic culture devices offer the methods for achieving 3D tissue culturing. Among those methods, culturing cells on biocompatible nanostructure scaffolds and multicellular organoids formation gained the most attention and showed high resemblance of an *in vivo* tissues [1, 86, 87, 89-93]. For example, Liz Y. Wu et al. have reported the use of micro-structures in microfluidic channels to trap multiple tumor cells and continuously culture them till the formation of solid multicellular spheroids (**Figure 2.5**) [1]. It is worth to mention the emerging 3D bioprinting technology which selectively plots living cells to construct tissues or organs. This technology could be a viable option in achieving organ *in vitro* models[94]. To date, several organ's function or disease models were successfully presented in microfluidic culture devices such as liver, kidney, lung, marrow, blood brain barrier and tumors [67]. A new research branch named organ-on-a-chip was generated aiming to produce highly imitate *in vitro* organ models [17, 67, 95]. Further micro-engineering of organs-on-a-chip devices focuses on developing more complex

multiple-organs system, which is more close to mimic the responses from human body. Interactions between each on-chip organ were transmitted by microfluidic networks that serve as a simulation of the human circulation system. Multiple-organ systems are also called human-on-a-chip, providing a promising image to futuristic individualised biomedical applications, especially for new drug discovery and clinical drug tests. However, microfluidic organ-on-a-chip must content with specific technical challenges that could impede to its practical applications. Firstly, fabrication requires highly specialized micro-engineering technologies. Secondly, the current organ-on-a-chip devices are fragile and their proper functioning requires careful maintenances. For example, continuous perfusion of culture medium by a pump is necessary in many organ-on-a-chip approaches, and even few bubbles in microfluidic channels may injure cells and hamper fabrication and control of chips, and it can be difficult to completely remove them. Thirdly, testing on the organ-on-a-chip devices strongly requires professional and bulky imaging instrument to analyse the results, which makes organ-on-a-chip devices hardly be used outside laboratory. Therefore, current organ-on-a-chip testing is difficult to conduct and time-consuming.

Figure 2.5: Microscope image of multicellular spheroids cultured in U-shaped microstructure [1]. (Image reproduced with permission from Springer)

2.3 Novel Design and Fabrication Technologies for Individualised Microfluidic Devices

Microfluidics originated from the semiconductor industry [20]. Using fabrication techniques identical with those in MicroElectroMechanical system (MEMS) technology, which has the capability to fabricate structures on the scale of micrometers and even nanometers [22]. To achieve small size fluidic components, including micro-channels, micro-reactors, micro-valves, micro-mixers and micro-pumps, MEMS microfabrication is required. As a typical example, poly(dimethylsiloxane) (PDMS) soft-lithography is the most widely employed in fabricating microfluidics devices (**Figure 2.6**). Fluidic networks on PDMS are created by casting PDMS in liquid form onto a hard-mould with negative patterns of designed structures, followed by high temperature curing of PDMS. On the other side, the mould is patterned by photolithography on a solid photoresist or directly etching on a substrate. Soft-lithography is convenient in design and fabrication, and PDMS based microfluidics devices have many advantages in biomedical researches since they are highly biocompatible, optically transparent and gas permeable [74]. Numerous applications have been demonstrated with the use of MEMS based microfluidics devices including cell manipulation, polymerase chain reaction (PCR), immunoassay and drug testing (**Figure 2.6**).

Recent developments using microfluidic applications in individualised medicine have brought new challenges to the design and fabrication of devices [12]. First of all, the massive employment of individualised medicine to patients would probably be applied in out-laboratory conditions and by non-medical specialists. One such example is the on-field diagnoses of infectious disease to patients in developing countries, or the monitor of patients' cancer metastasis in small clinics [10, 16, 21, 96]. Unfortunately, most of currently available microfluidics devices required sophisticated and costly laboratory equipment and well-trained

Figure 2.6: Soft-lithography processes with PDMS and SU-8 photoresist to fabricate microfluidics channel. 1. Spin coat SU-8 photoresist. 2. UV exposure to pattern the photoresist. 3. Developing to release the mould structure. 4. Cast PDMS on the mould. 5. Peel cured PDMS from the mould and paste on a layer of PDMS to complete the fabrication of PDMS microchannels.

specialist personnel. Independent test-analyse-report microfluidic devices that integrate multiple functional components on the same platform can replace complex laboratory equipment and manual operations.

Secondly, the diagnosis and treatment must be individualised for individual patient, and in order to meet this requirement customized medical products become necessary [2, 97]. However, although the fabrication of microfluidic devices is convenient to researchers with sufficient facilities, the cleanroom-based microfabrication techniques are hardly accessible to most end-users like doctors and patients [2]. Therefore it is almost impossible to modify microfluidic devices based on each patient's condition. Therefore a flexible design and modular fabrication of microfluidic devices must adopted to significantly enhance the capability of microfluidics devices to deal with various and complicated situations, which would be usually encountered in individualised diagnosis and treatment[2, 97-99]. Furthermore, customized medical products can be available to every patient only if the cost in

design and fabrication would be reasonable. In a word, novel fabrication methods must be developed to replace MEMS cleanroom-based microfabrication.

In this section, such novel design and fabrication technologies for microfluidic devices towards individualised medicine are discussed. The independent test-analyse-report microfluidic devices become possible only with the recent advances of system integration and realization of lab-on-a-chip platforms, in which dedicated functional components were integrated together with microfluidic and other types of devices. The potential in broadening the employment of microfluidics devices in a large range of applications and situations have been seen. Moreover, facing the challenge on customizing medical devices based on patient-tailored specifications, modular microfluidic blocks were introduced to achieve device's flexibility and reconfiguration in individualised application. In the last part of this section, recent progress of 3Dimensional (3D) printing microfluidics device is reviewed as this become a key fabrication technology which is believed to be more suitable for the implementation of devices for individualised medicine.

2.3.1 Independently Test-Analyse-Report Microfluidic Devices

Individualised medical devices shall be tailored according to be used on patients' needs and the flexibilities in usage so as to be capable to perform the necessary specific treatments for the target patients. Unfortunately, most of the currently developed microfluidic devices strongly rely on laboratory equipment and need to be operated by well-trained staffs. And their setups usually required syringe pumps and tubes to manipulate micro-fluids (**Figure 2.7**). Not only the bulky and expensive features for professional syringe pumps, but also the low pumping efficiency of this method in which one pump can only manipulate the flow in only one channel impede its employment in future medical applications. Besides fluid manipulation, microscopy is one of the most important tools for biomedical tests and it

becomes even more necessary when those tests are miniaturized in microfluidic devices. Therefore, the accuracy of microfluidic tests is strongly determined by microscopy and associated image analysis, but currently those processes are only conducted with microscope and a PC. Efforts have been spent to overcome the excessively relying of additional resources for performing standalone microfluidic applications.

By integrating actuation components in microfluidics device, fluid manipulation without additional external instruments becomes possible. Micropumps have small size, low power consumption and high accuracy and they were being used for microfluidics. There are generally two types of on-chip micropumps: the active and the passive. Active pumps transfer energy into forces to move fluids. As a typical example, piezoelectricity, the phenomenon that changes the shape of a piezoelectric material when an external voltage is applied. It has been widely used in designing MEMS micromotors and micropumps [100]. For example, Xu et al. have demonstrated the use of a piezoelectric pump as the actuation of multiphase microfluidics[101]. Electromagnetic actuation is another principle on which micropumps can

Figure 2.7: Microfluidics lab-on-a-chip device developed for long-term bacteria culturing. External tubes and syringe pumps were added to the device for fluids manipulation[3]. (Image reproduced, with permission from American Association for the Advancement of Science) microstructure [1]. (Image reproduced with permission from Springer)

be built and operated[102]. Recently a microfluidic product named IPRECIO drug infusion pump become commercially available on market. It is equipped with integrated electromagnetic rotation micropump that has been used in several implanted drug treatment studies [62, 66]. Instead of transferring energy to displacement of materials, the category of active pumps named electrochemical micropumps generate forces by inducing electrolysis of fluids, which transfer liquid phase water into gases (Oxygen and Hydrogen) and achieves high pressure to drive fluids in the microchannels. Electrochemical micropumps were chosen by us as well as other research groups who use them in several lab-on-a-chip and implantable drug delivery device studies [9, 13, 48, 65]. This choice is justified by the important advantage of this pump on simple structure, small size and high pumping efficiency (**Figure 2.8**).

Passive micropumps represent another solution to control the fluid flow in microfluidic devices, due to the simplicity in the implementation and fabrication of the system [103, 104]. Finger pressing forces [32], capillary [104, 105] and gravitational [106] forces are being studied to provide fluid flow manipulation. For example, Li et al. have demonstrated a capillary pump integrated on a lab-on-a-chip device for blood tests[104]. Passive driven fluid flow is ideal in the design of individualised medical microfluidic devices because it totally removes the need for external instruments and external power supply. However, passive micropumps have limited control over the flow rates which cannot reach large values thus it result in long period of time to be consumed for detecting desired biomolecules.

Besides micropumps, imaging components were also successfully integrated into microfluidic devices, especially in lab-on-a-chip applications. For example, Moon et al. and Ozcan et al. [107, 108] have presented a lensless shadow imaging technique to observe cells and acquire images with CCD/CMOS detectors integrated together with the microfluidics.

Figure 2.8: A stand-alone lab-on-a-chip device equipped with electrochemical micropumps: **a.** Photograph of the device. **b.** Photograph of the integrated electrochemical micropump[8].

High resolution cell imaging, called “microscope on chip”, was demonstrated by Cui et al [109]. Indeed, with the great development of built-in cameras on smartphones or tablets, there is indeed a potential in using such compact imaging device in individualised medicine. Tseng et al have presented a lensfree microscopy technique installed on a cellphone [110]. Zhu et al. also designed a smartphone imaging system for microfluidics chip (**Figure 2.9**) [5]. Furthermore, the image processing and result analysis were accomplished by using software available in the smartphone’s operation system (Android by Google). It is also worth to mention that cellphones are connected to Internet thus making it easy for result analysis to be send to and read by a doctor or a technician online located anywhere in the world instead of being beside the tester. The cooperation between microfluidics devices and smartphones provides a meaningful and fascinating prospective for developing individualised microfluidics devices which can easily substitute conventional instrument as well as technical manpower.

2.3.2 Modular Programmable Microfluidics Devices

Figure 2.9: Fluorescent imaging cytometry system on a smartphone [5]. (Image reproduced with permission from American Chemical Society)

However, despite having more and more microfluidic devices being employed in healthcare applications in recent years, unexpected challenges appeared to both developers and end-users. First of all, individualised medicine requires highly customized design but at low volume production of each specifically designed device [97]. Moreover, current design and fabrication protocols for microfluidics devices involve highly skilled manpower as well as expensive micro-fabrication methods. Therefore, individualised microfluidic devices are not cost-effective presently and can be widely used only if the costs are cut down substantially. Secondly, the requirements for each type of microfluidic device are directly originated from end-users, who are biologists, doctors and patients. But the lack of MEMS engineering knowledge makes the modifying of pre-designed microfluidics products to fit end-users' needs impossible [2, 99]. For example, immunoassays of infectious disease requires variable types on reactions, thus different designs of reaction chambers, microfluidic networks as well as the types of reagents must be carefully chosen in each test. Existing immunoassay chips usually have a fixed target thus they have no flexibility in usage, which

increases the difficulties to modify such devices or to understand the working principles of each device.

Microfluidic device developers proposed modular designs for the products to be used individualised medicine [2, 98]. Instead of surface micro-patterning all passive fluidic components and actuators on a monolithic platform, the idea proposes that discrete components can be fabricated separately and finally be assembled by end-users based on their needs. There are basically two approaches for realizing modular microfluidics devices. The microfluidics breadboard (FBB) was the first one to be introduced. Similar to the electronic breadboard which provides connections to each electronic component (resistors, capacitors, LED etc.), FBB provides microchannels to link other fluidic components including reaction chambers, mixers, valves, and pumps; sensors on flow rate, optics, temperature and pressures; other functional components like heaters and lasers [97, 98, 111]. Customized microfluidic devices then can be easily built and programmed by plotting components on FBB through hole/via on the FBB. For example, Shaikh et al. have demonstrated different biological applications on one FBB by slightly changing connected components [97].

In another modular microfluidic approach, researchers removed the need of FBB by

Figure 2.10: Discrete components to build 3D modular microfluidics device [2]. (Image reproduced with permission from the author)

designing universal connectors on each element, hence these elements can be directly connected to each other [2, 98]. Compared to the FBB approaches, method with direct connections has less limits regarding the system size, thus this design could benefit applications with complicated microfluidics design. For example, Bhargava et al. have presented a 3D microfluidic circuit that makes use of discrete components in digital droplet manipulation (**Figure 2.10**) [2]. As a disadvantage, the installation of microfluidic networks without FBB can only be done by someone with appropriate know-how and skills. It must also be noticed that modular microfluidic devices offer great flexibility in multiple functions but the capability of each device is strongly determined by the size of the components library. Therefore, the development of microfluidics could refer to the previous development of electronics [112], and a universal protocol of designing modular microfluidics device may need to be proposed and implement in the future to enable wide and easy use of fluidic components fabricated by any company.

2.3.3 3D Printing Microfluidics Devices

3D printing, also named additive manufacturing, is a fast growth technology of great development in many areas. Three-dimensional objects are printed by duplicating multiple 2-D successive layers in the vertical direction finished by an industrial robot called 3D printer. Nowadays 3D printing offers a user-friendly environment to build complex products which can be designed with the use of Computer Aid Design (CAD) programs. In comparison to traditional manufacturing, 3D printing is extremely low-cost and it requires fewer manpower or material resources. With higher resolution 3D printing machines currently available (tens microns in plane resolution and few microns in vertical resolution), their applications in microfluidics have merged in recent years with a number of applications being demonstrated [113-119]. 3D printing seems highly suitable to microfluidic devices for individualised

medicine, since highly customized biomedical products can now be easily printed at an affordable cost no matter how large is the volume of production. Moreover, for existing modular microfluidics platforms, 3Dprinting of new components based on specifics need would certainly enhance their capability in dealing with highly flexible applications.

The implementation of 3D printing in microfluidics commonly includes two approaches. In the first approach, the fabrication processes are similar to traditional soft-lithography but with 3D printed moulds [115, 116]. For example, Kamei et al. have presented a cell concentration microfluidics chip through PDMS soft-lithography on a 3D printed mould[116]. Unlike a mould patterned by hard photoresist photolithography, 3D printed mould has higher design range in the vertical direction, and can also achieve irregular shapes which can hardly be fabricated by photolithography. This approach can be seen as an update to the widely used fabrication method, thus it can be easily accepted by microfluidics researchers, but its significance for individualised medicine is limited. In the second approach, the complete device is solely fabricated by 3D printing and thus it would be more useful in individualised medicine. Practical applications like 3D printed implants have been successfully demonstrated that showed the potentials. Challenge of this approach may be the fabrication of actuators and controllers, which contain materials beyond the available materials in 3D printing technologies, especially metals. It is worth to mention a recent emerging technique called 3D bioprinting, which intends to print tissues and organs. Living cells, supporting structures and other biomaterials are printed into tissues and organs majorly considered for their transplantation in current development [94]. The author of this thesis would like to draw the attention on the future application of this technology for individualised medicine. 3D printed *in vitro* organs and tissues would serve as perfect models for drug discovery and test. Moreover, the border of implantable devices will be greatly expanded if

living cells are considered as materials to construct those devices. Especially drug delivery devices could be replaced drug continuous production-delivery of man-made organs.

2.4 Motivation and Objective

Implantable drug delivery devices are viewed as an approach for more controllability and flexibility in drug treatment since they demonstrate the method to overcome the disadvantages of conventional drug delivery routes, including oral pills and capsules, intravenous therapies and syringe injections. In our proposed study, an implantable drug delivery device can be used in cancer chemotherapies, during which patients suffer from serious side effects from the oral or intravenous administration of chemotherapeutic drugs, it is hoped that this new generation of advanced chemotherapy will be made possible by implantable drug delivery devices due to their ability to greatly enhance the efficiency of drug delivery. However, there remain many unanswered questions regarding to the actual usage of such device, such as the therapeutic effects of localised chemo-drug delivery is not clear; the biocompatibility of the device must be guaranteed during the implantation; and the reliability of devices' working after the implantation. We aim to develop a MEMS implantable drug delivery device and investigate the effects of locally treating chemo-drugs using this device. Next, we aim to test this device *in vivo* in small animals for comprehensively understand the device's biocompatibility and working reliability under the implanted settings.

In individualised cancer treatment, the therapy must be specifically tailored for every person thereby maximizing the therapeutic effects while minimizing the potential side effects. However, in order to determine an optimized treatment plan for each patient, a large number of tests are required to evaluate the effect of different drug formulations and treatment plans per individual, prior to commencing the actual therapy plan. LOC devices offer an attractive

solution to these challenges through the miniaturization and automation of the testing processes. The shortcoming of these LOCs however, is that they are configured to perform miniaturized parallel versions of various tests to collect data on the treatment response on a patient's cell samples. Therefore a bulk testing condition is used to determine an individual response, where in reality the physiology and biology of each patient has great variation. This limitation stems from the overreliance of current LOCs on large off-chip components such as pressure pumps for fluids operation, making customization for individualised testing unfeasible. Such obstacles will limit individualised approaches from being applied on a clinical scale where there are multitudes of permutations to test for every individual and this usually requires an unrealistic number of these large off-chip components. Standalone LOC systems are ideal solutions to overcome these challenges as they integrate highly customizable modules, containing all the required components for operation, potentially allowing individualised testing for individualised treatment. Therefore, we aim to develop the platform utilizes biocompatible functional modules to replace the large off-chip components in current LOC systems, such as large fluid pumps, control systems, etc. Next we aim to merge the LOC system with cancer cell-on-a-chip and even tumor-on-a-chip techniques for the anticancer drug test *in vitro*, LOC technologies help anti-cancer drug researchers to establish advanced *in-vitro* testing platforms thereby the performance of the treatment can be easily and carefully evaluated. We aim to achieve high-throughput, ease-of-use, high accuracy, low cost drug test using the device for the optimization of cancer treatment.

CHAPTER 3 THE DEVELOPMENT OF MEMS IMPLANTABLE DRUG DELIVERY DEVICES FOR LOCALIZED ADMINISTRATION OF CHEMOTHERAPY

Implantable drug delivery devices are viewed as a next-generation solution for more controllability and flexibility in drug treatment since they could overcome the disadvantages of conventional drug delivery routes, including oral pills and capsules, intravenous therapies and syringe injections [9, 30, 45, 48]. They are of particular importance in cancer chemotherapies, during which patients suffer from serious side effects from the oral or intravenous administration of chemotherapeutic drugs, it is hoped that a new generation of advanced chemotherapy will be made possible by implantable drug delivery devices due to their ability to greatly enhance the efficiency of drug delivery [9]. In our proposed chemotherapy technique with an implantable device, a minimally invasive surgery is firstly required to place the microdevice near the tissues with tumors to allow localized administration of therapies. Chemotherapy is then initiated by activating the device's delivery function. Chemotherapeutic drugs are only delivered into tumor areas, so their toxicity to normal organs and tissues is minimised when they are compared to conventional treatments of drug. Also, a long-term device implantation can be used to accomplish continuous tumor inhibition. Eventually, the device can be removed from the body by means of a simple surgery when the treatment is accomplished.

Furthermore, localized drug delivery to a tumor by means of an implantable device greatly increases the resolution of drug performing. Modification of the device allows precise tailoring of drug formulations, treatment times and treatment periods [12, 48]. This feature allows doctors to develop detailed and flexible therapeutic strategies (e.g., pulses and continuous delivery) to treat complex diseases such as cancer. From the perspective of

individualised medicine, a customisable treatment is most suitable and most effective because of its ability to define treatment by closely meeting the needs of each patient.

In this chapter, we present an implantable drug delivery device that was designed for localized administration of chemotherapy. The developed device includes a single reservoir with an integrated micro-actuator. Electrochemical pumping was used for actuation and was realised by the placement of interdigitated Pt/Ti electrodes inside the device. The entire device was fabricated by means of MEMS microfabrication, including photolithography, soft lithography and surface micromachining. We first demonstrated the use of the MEMS drug delivery device for individualised drug therapy *in vitro*. The results highlight the importance of customising a specific drug delivery profile for the treatment of different types of pancreatic cancer cell lines. We then demonstrated an *in vivo* evaluation with Kunming mice. We also carefully examined the biocompatibility and working reliability of the devices during a subcutaneous implantation into mice for 28 days.

3.1 Design and Fabrication of MEMS Implantable Drug Delivery Device

3.1.1 Design

The developed device was designed based on a single reservoir with an integrated actuator. As discussed in Chapter 2.1, the single reservoir design in drug delivery micropump enables refills of the drug reservoir. Therefore these device is more suitable in a long term chemotherapy because it avoids periodical surgeries to replace the implants [48]. Moreover, the ability to modify loaded drugs is of great significance in dealing with cancer, in which a strong resistance can be rapidly built up to the administered medication [58]. Thus, combinations of drugs as well as frequent amendments of formulations over time are generally needed to be employed to counter these resistances thus the cancer treatment can be more appropriate and effective [9]. Following the design, our MEMS implantable drug delivery device was constructed with three major parts. They include a refillable drug reservoir, a long cannula, and a Pt/Ti electrode actuator for pumping (**Figure 3.1**), respectively.

The drug reservoir and cannula were built using PDMS due to its tested highly biocompatibility [71]. Likewise a PDMS-based reservoir has the advantage that it can

Figure 3.1: Schematic view of the 3 major parts of the MEMS drug delivery device.

Figure 3.2: Illustration of the subcutaneous implantation: The device's long cannula is extends to disease area and the implanted device can be easily refilled by using a syringe.

inherently self-reseal from poking [120], While at the same time preventing any drug leakages from the reservoir. Therefore, this essential property enables easy refilling by using a standard syringe and needle, for example when such device is subcutaneously implanted (**Figure 3.2**). The long cannula enables drug flowing to targeted treatment areas. Hence the whole device does not have to be placed right at the target but somewhere in its vicinity, allowing the user to choose a suitable and convenient implantation area. The PDMS cannula is soft and flexible, thus it can be safely placed inside tissues without creating any discomfort or impairing any body movements. The device's fluidic actuator contains a pair of interdigitated Pt/Ti electrodes at the bottom of the drug reservoir for pumping. The electrode actuator features both a small size and high pumping efficiency, and has been applied in several implantable drug delivery approaches [29, 42, 65].

3.1.2 Working Principle

The MEMS implantable drug delivery device utilizes electrochemical actuation. When a D.C. bias voltage is applied onto the actuator, electrolysis takes place at the interdigitated Pt/Ti electrodes. The arising electrolysis reaction breaks down the liquid water into oxygen collected as a gas at the cathode, and hydrogen appearing as a gas at the anode. These generated gases pressurize the drug reservoir and push its contents out through the long

Figure 3.3: Schematic of the electrode actuator and the working principle of its electrochemical actuation

cannula (**Figure 3.3**). The actuation is terminated by simply with removing the applied bias voltage.

3.1.3 Fabrication Processes

The drug reservoir and cannula were fabricated using PDMS soft lithography on a SU-8 photoresist mould (**Figure 3.4**). The mould's pattern was drawn with using a computer-aided design software (L-EDIT) and then the pattern was printed on a photomask (chromium patterns on soda lime glass). SU-8 positive photoresist (Gersteltec Engineering Solutions, Pully, Switzerland) was patterned onto the Si substrate (LATECH Scientific Supply, Singapore) by spin-coating at 500 revolutions per minute(rpm) for 60 s to achieve a 500 µm thickness, followed by a pre-exposure bake (110 °C, 1 hour). Photolithography transferred the pattern from the photomask onto the SU-8 photoresist (using ultra-violet light of 365 nm wavelength, 420 W, 90 s, hard contact), followed by a post exposure bake (95 °C, 1 hour). Finally, developing was performed to release the SU-8 mould by chemical etching away unexposed SU-8. Polydimethylsiloxane (Sylgard 184, 10:1 base to curing agent ratio, Dow Corning, Michigan, USA) was mixed and poured onto the SU-8 mould. Then the sample was degassed inside a vacuum desiccator to remove all air bubbles. At last, the PDMS on the SU-

Figure 3.4: The fabrication processes of the drug reservoir and cannula: **a.** Cross-sectional view of the process. **b.** Schematic view of the fabrication flows. 1. Silicon wafer. 2. Spin coating SU-8 onto the silicon wafer. 3. Photolithography. 4. Developing. 5. Pouring PDMS on mould. 6. Curing PDMS and removing from mould. 7. Finished PDMS reservoir and cannula.

8 mould was cured at 120°C for 20 min on a hot plate and was subsequently removed from the mould to obtain the drug reservoir and cannula structure.

The electrode actuator was fabricated by surface micromachining on a silicon wafer (**Figure 3.5**). Interdigitated Pt/Ti electrodes patterns were drawn with computer-aided design software (L-EDIT) and then the pattern was printed on a photomask (chromium patterns on soda lime glass). AZ5214 photoresist (AZ Electronic Materials, Branchburg, NJ) was spun coated onto the Si substrate at 4000 rpm for 45 s to achieve 1400 nanometers. The sample was baked before exposure (105°C, 2 min) to evaporate solvents. The electrodes' pattern was

transferred onto the wafer by photolithography under a mask aligner (model MA-6, Suss MicroTec, Germany) and a reverse baking on a heat plate (120°C, 2 min). The generated pattern was then developed by the wash of the AZ developer (Branchburg, NJ).

The actuator's metal structure includes layers of titanium (Ti, Ted Pella, Inc. USA) as adhesive layers, and platinum (Pt, Ted Pella, Inc. USA) as functional layers, which were deposited by electron beam evaporation to create the electrode structure. The remaining AZ photoresist was lifted off thus the electrodes were released. A pair of thin copper wires (0.1mm in diameter, RS Component, SG) was bonded to anode and cathode of the electrodes with silver conductive adhesive paint (RS Component, SG) to enable the connection with the

Figure 3.5: Fabrication processes of the electrode actuator. **a.** Cross-sectional view of the process. **b.** Schematic view of the fabrication flow: 1. Silicon wafer. 2. Spin coating AZ5214 on silicon onto the wafer. 3. Photolithography. 4. Developing. 5. Electron-beam Titanium evaporation. 6. Electron-beam Platinum evaporation. 7. Lift-off. 8. Cutting unused silicon.

external power supply.

Eventually, the three parts were assembled together by using PDMS as adhesive agent. The finished device (in its latest version) was 2 mm thick, 10 mm long, and 10 mm wide drug reservoir and bonded with a 40 mm long cannula. In summary, the whole fabrication processes involve few steps of common clean room techniques and use inexpensive materials. Therefore, the device design is suitable for future mass production. It is also worth to mention that the soft-lithography which builds drug reservoir and cannula could be replaced with 3D printing in the fabrication of moulds to further reduce the number of processes and cost.

3.1.4 Characterization

The electrochemical actuation employed by our device has the ability to control the pumping flow rate, which has been demonstrated in several studies [45, 65]. Basically a higher bias voltage would result in a higher pumping efficiency, resulting in a higher delivery flow rate. The actuator's actual pumping flow rates under different bias voltages was

Figure 3.6: **a.** Schematic view of the device after assembly. **b.** Photograph of a fabricated device.

Figure 3.8: Flow rates vs external bias voltage for our MEMS drug delivery device

($N=5$, mean $\pm$ SD).

measured (3, 4, 5, 6, 7, 8 and 9V) (**Figure 3.7**) for our developed drug delivery device. Theoretically, the water electrolysis could proceed under a direct current (D.C.) bias voltage that is higher than 1.23 V (overpotential). In our measurements, the electrochemical reactions can visibly be observed when a minimum 3V bias loaded. Therefore, we have measured the pumping performance at 3-7 V in our early designed devices [48]. However, when we further miniaturized the device for *in vivo* usage with small animals, we observed that the smaller actuator provided dramatically lower pumping rates, which were less than 1 $\mu\text{L/s}$ as well as bad device responsivity (e.g. it took more than 5 minutes to detect the first drug droplet being delivered). Therefore, considering the actual applications of our device, we have set the minimum bias voltage to be 5V, which gives a small flow rate and fast responsivity. For higher bias voltage than 9V, the Joule heat instead of electrolysis will dominate the actuation process. In our observation, devices' pumping flow rate at 10V become unpredictable because the heat accumulation can lead to a dramatically increase of drug delivery speed. To avoid the unpredictable delivery flow rate as well as the potential negative effects due to the temperature increase in drug reservoir, we have set the maximum bias voltage to be 9V. A

constant D.C. voltage source was used to supply the desired bias voltages to the tested devices under a room temperature of 20°C.

The drug reservoir of each device was filled with DI water and refilled in-between each test using a syringe (30 Gauge needle). Power was switched on for one minute and the solution that flowed out from the device during this period was collected into a plastic tube for weighting. The weights of the collected DI water were then calculated into volumes (1 microgram per microliter), thus the measured flow rates data could be calculated and plotted as delivered fluid volume versus time in the unit of $\mu\text{l/s}$ for each applied bias voltage.

The obtained results, after averaging over 5 samples ($N=5$) are shown in **Figure 3.8** as mean flow rates with standard deviations. An approximately linear relationship between bias voltages and delivery flow rates can be observed ($\text{Flow Rate} = 0.356 \times \text{Voltage} - 0.834$, $R^2=0.98$). Therefore, delivery dosages can be controlled by choosing the appropriate bias voltage and operation time. This feature would help the users to define a flexible therapeutic plan for individual patients, with the benefit of high accuracy and controllability. Additionally, the electronic operation of the device could be easily simplified by using a controlling circuit or computing device in which program of drug delivery parameters could

Figure 3.7: Drug delivery characterization experimental set-ups.

be loaded and run, to enable automatic operation of the device.

3.1.5 Electrochemical Actuator Optimisation

As the available animal models are commonly limited to simulate the actual implantation area, the MEMS implantable drug delivery device must be further miniaturized for *in vivo* studies. However, challenges occurred when the size of the electrochemical actuator was also shrunk. It was observed by several research groups that when the feature size of the interdigitated electrodes decreased to be less than 100 micrometers, the lifetime of the actuator would be significantly shortened, showing a sudden drop in pumping efficiency or even exhibiting a totally pumping failure within a short period. This shortcoming greatly affects the reliability of the device. Unexpected fluctuations of pumping efficiency would also lead to an unpredictable delivery flow rate, which may cause unsuccessful treatment due to the delivery of either an insufficient amount of drug being or an overdose. Moreover, after its complete actuator failure, the whole implanted device must inevitably be replaced through surgery.

Studies have shown that metal delamination from electrodes was the major reason for the electrochemical actuator failure [29, 65]. Such delamination is due to the forces caused by the forming and burst of air bubbles. For the commonly used two-layer electrode design that contains an adhesive layer and another functional layer, a small bias current (5 mA) can initiate the metal delamination [65]. To comprehensively understand the electrode actuator damages due to a rapid and intense electrochemical reaction, we utilized scanning electron microscopy (SEM) to investigate the actuator failure mechanism. A Ti/Pt electrode actuator was fabricated as mentioned above, with the electrodes patterned to have the uniform finger width (20 μ m) and finger spacing (20 μ m). Each sample was biased at a constant D.C. bias voltage of 8 V while immersed in DI water for a continuously actuation. The electrodes were

Figure 3.9: **a.** SEM image showing metal delamination from electrode fingers after continuous electrolysis at 8V bias for 2 minute. **b.** 4 minutes. **c.** A delaminated metal piece short-circuits two adjacent electrode fingers after 2 minute operation. **d.** The same region after 6 minutes actuation. All scale bars represent 20 μ m.

then imaged under the SEM at every 2 minute intervals. Our test results suggested that the metal delamination occurred within the first 2 minutes (**Figure 3.9a**), and the damages repeated with creating new empty regions on several metal electrode fingers (**Figure 3.9b**). We concluded that the break-down at the electrode fingers was the possible reason of actuation efficiency dropping as the reaction area decreases significantly. It was also observed that delaminated metal pieces could be larger than the gap between electrode fingers. Therefore they could short-circuit two adjacent fingers (**Figure 3.9c**). We have also confirmed that the attached “metal bridge” did not disappear by further actuation (**Figure 3.9d**). These short circuits of the actuator’s metal fingers could lead to immediate actuator failure, or dramatic temperature increasing. It can be predicted that as the electrodes’ spacing

Figure 3.10: SEM image showing the nanosandwiched Pt/Ti multi-layers deposited on silicon.

decreases for devices miniaturized at smaller feature sizes, the risk of short circuits increases dramatically.

To overcome this problem, Sheybani et al have introduced the use of a thin Nafion layer coated on their surface of the electrode actuator as a protective layer to reduce the physical damages onto electrode fingers [121]. In our study, for the 2nd, improved, version of our device, we created a nanosandwiched Pt/Ti multi-layer electrode design to overcome metal delamination thereby to enhance the device's reliability. In the multilayered design, the metal finger was fabricated with a sequence of units of thin Ti/Pt layers (100nm Ti and 60 nm Pt in thickness), which were vertically deposited on the substrates, and a relatively thick layer of Ti (600nm in thickness) was then placed on the top of the electrode as a protective cap (**Figure 3.10**). Compared to conventional two-layer electrode design, the nanosandwiched Pt/Ti multi-layer electrode provides strong physical protection to the Pt layers by enclosing them inside the protective Ti layers in order to avoid their delamination. **Figure 3.10** shows the cross-sectional SEM image of the nanosandwiched Pt/Ti multi-layer electrode actuator. Platinum film plays a more important role than titanium film in electrochemical actuation because it withstands oxidization. Therefore, Platinum film's lateral areas are considered as the reaction areas in the electrodes.

Figure 3.11a. SEM image of metal electrode fingers at 8V D.C. bias after 12 minutes actuation. **b.** Close SEM image of metal electrode fingers at the same bias after 30 minutes actuation showed slight damages. All scale bars represents 20 μm .

The nanosandwiched Pt/Ti multi-layer electrode actuator was also tested under a constant D.C. 8V bias voltage. The strength of the new design was confirmed by observing no obvious damage of the metal electrode structures even after 12 minutes of actuation (**Figure 3.11a**). Thus the short circuits were avoided and no large metal pieces delaminated.

A stress test was performed subsequently to further understand the nanosandwiched Pt/Ti multi-layer electrode actuator's performance under extreme conditions. **Figure 3.11b** shows that after 30 minutes of continuously operation, slight damages on metal electrode fingers appeared. However, unlike the delamination of large metal pieces in the conventional two-layer design, the damages of the new design would only generated only small metal fragments, which were not large enough to bridge two adjacent fingers and short-circuits. When the actuation time increased to more than 30 minutes, the small damages on metal fingers would accumulate, until they eventually the actuation stopped completely. In conclusion, the nanosandwiched Pt/Ti multi-layer electrode actuator is able to withstand electrochemical damages on its metal structure for a much longer time, thus exhibiting longer operational lifetime than the conventional two-layer electrode actuator. In applications, this contributes to a significantly increased reliability over a long period.

For the reliability evaluation, the *Mean Time To Failure* (MTTF) of devices from different electrode groups (with 6 devices in each group) were measured. For each device, the measurement was performed in a test structure where the flow rate of the device was measured with respect to time. A constant voltage of 8 V was supplied throughout the tests. The “*Time to Termination*” was set to 500 s, i.e., the flow rate of each device was monitored for 500 s. The device was treated as a “*Failure*” when the flow rate drops to 80% of the initial value, where the time was termed the “*Failure Time*” of this device. Devices which did not fail at the end of the test were treated as “Censored”. The *MTTF* value is calculated as:

$$MTTF = \frac{\sum(t_{Failures}) + n_{censored} \times t_{Termination}}{n_{failure}}$$

Where $t_{Failures}$ represent the failure time of each failed device, $n_{censored}$ represents the number of censored devices, $t_{Termination}$ represents the time to termination of the test and $n_{failure}$ is the number of failed devices after the test. In an ideal case, the flow rate of a device is expected to be constant under fixed voltage values. However, in reality, at higher voltages, the flow rate drops as delamination occurs. For our case, a sample is considered to have failed when its flow rate drops below 80% of the initial flow rate. In the test, flow rates of six electrode samples in each of the three groups (conventional two-layer design on Si, nanosandwiched multilayer design on Si and nanosandwiched multilayer design on SiO₂) were monitored for 500s (Time to Termination) and the results are shown in **Figure 3.12**. For the conventional two-layer design group, all six devices failed the test, with a calculated MTTF of 5.2 min. As for the other two groups, each had only 2 devices failed in the test and the remaining 4 were censored (Type I censoring). The MTTF calculated for the nanosandwiched multilayer design on Si and SiO₂ are 24.3 and 23.0min, respectively. These results show that the reliability of the nanosandwiched multilayer design, regardless of the substrate, is much better than the conventional two-

Figure 3.12: Mean Time To Failure (MTTF) measured for electrodes fabricated from different designs, i.e., the conventional two-layer design on Si substrate, the nanosandwiched multilayer design on Si substrate and the nanosandwiched multilayer design on SiO₂ substrate. Sample size $n = 6$. The Time to Termination is set to be $t = 500$ s and samples that did not fail within 500 s were treated as Censored. The two groups of electrodes with nanosandwiched multilayer design both have 4 samples censored.

layer design. It is important to note that the MTTF of the nanosandwiched multilayer electrodes were obtained under a high stress condition of 8 V. Therefore, under normal operating conditions (~ 5 V), we can expect that the MTTF of the nanosandwiched multilayer electrodes to be much longer than 20 min. It has been reported that small electrode finger width and spacing of less than $50\mu\text{m}$ in a conventional design would lead to severe metal structure damages and actuator failures. In a summary, our new design provides a promising approach for fabricating highly reliable electrodes with much smaller finger patterns of $20\mu\text{m}$ and overcoming the challenges mentioned above.

Figure 3.13: Flow rates characterization of nanosandwiched electrode actuator, compared to conventional 2-layer design. “C” stands for “Conventional design” and “M” stands for “Multi-layer design”

We have also characterised the delivery flow rates of the novel nanosandwiched Pt/Ti multi-layer electrode actuator. First, we compared the multi-layer actuator with a single Pt layer on Ti (100 nm Ti as adhesive, 60 nm Pt as functional and 300 nm Ti as cap layer) and with the conventional two-layer metal actuator (100 nm Ti as adhesive and 60 nm Pt as functional layer), both with the same thickness of the Pt layer. It can be noted from **Figure 3.13** that the multi-layer actuator had a lower delivery flow rate than the two-layer actuator at all bias voltages. To explain this phenomenon, energy dispersive spectroscopy (EDS, OXFORD Instrument) was being used to analyse the differences between the two designs. EDS indicated oxidations occurring on the multi-layer electrodes after 2 minute actuation while the two-layer actuator remained un-oxidized. As titanium is more active than platinum, we suggested that the lower pumping efficiency that was related to the oxidation of the Ti cap

Figure 3.14: Flow rates characterization of nanosandwiched electrode actuator with two Pt/Ti units on SiO₂ and Si surfaces, compared with conventional 2-layer design. “C” represents conventional design, “S” represents nanosandwiched design on silicon dioxide and “M” represents nanosandwiched design on silicon.

layer on the multi-layer actuator. The forming isolation TiO₂ layer subsequently stopped the electrolysis at the electrode surface and left the reactions carrying only on lateral platinum surface on the electrode wall. Compared to the conventional design, the reaction areas on the multi-layer electrode are significantly smaller thereby the pumping efficiencies are lower. **Table 3.1** shows the calculation of Pt functional areas in each electrode design on an electrode finger. Obviously, conventional design has a much larger functional areas. However, it is interesting to notice that the pumping efficiency in conventional design is not as much larger than nanosandwiched designs as the functional areas differences among them. The mechanism leads to this phenomenon is still unknown to us, further studies is strongly needed to understand this phenomenon.

It is definitely possible to increase the reaction areas in multi-layer electrode by increasing the areas of Pt functional layer. In our nanosandwiched design, adding more units of Pt/Ti layers or increase the amount of Pt in each unit would help to enhance the pumping rate. Our characterization showed that with the unit number increasing from $n=1$ to $n=3$, the delivery flow rates under each bias voltage increased respectively (**Figure 3.15**). Besides, the

Design	Approximate Linear Function	R ²
C (60nm)	$y = 0.25x - 1.00$	0.998
M (n=1)	$y = 0.10x - 0.50$	0.995
M (n=2)	$y = 0.15x - 0.65$	0.977
M (n=3)	$y = 0.16x - 0.67$	0.992
S (n=2)	$y = 0.22x - 0.95$	0.950

Figure 3.15: Linear regression of the characterization results.

linear relationship between supplying voltages and flow rates remained in the pumping by the nanosandwiched multi-layer electrode actuator (**Figure 3.14**).

As we mentioned above, the electrolysis reactions are carried out on the electrode's wall instead of its oxidised surface thus it requires water transport into the gap between two adjacent electrode fingers. Under these circumstances, the surface hydrodynamic profiles play an important factor in the actuation efficiency. It has been demonstrated that the more hydrophilic the substrate, the easier of the mass transport of water to the electrolysis reaction areas[122]. To investigate the effects on the multi-layer electrode actuator, we fabricated the multi-layer electrode actuator on silicon dioxide (SiO₂) substrate and characterised its pumping performance. The test results indicated that with the same number of Pt/Ti units (n=2), actuation performed more efficiently on SiO₂ surface than on Silicon (**Figure 3.14**). We suggested the higher actuation efficiency was due to the more hydrophilic SiO₂ surface [123].

Design	Pt Functional Areas (μm^2)
Conventional Two Layer Design	2012.24
Nanosandwiched Design, Pt/Ti Unit No.=1	12.24
Nanosandwiched Design, Pt/Ti Unit No.=2	24.48
Nanosandwiched Design, Pt/Ti Unit No.=3	36.96

Table 3.1: Calculation of the Pt functional areas in each electrode's design assuming the electrode's size is 100 μm long, 20 μm wide.

In conclusion, a novel nanosandwiched multi-layer electrode actuator design was introduced in order to overcome the metal delamination in the electrolysis reactions. Compared to the conventional 2-layer design, the new multi-layer electrode actuator showed a more robust performance in actuation. Also it capably controlling the delivery flow rates and gained the possibility to enhanced delivery rates by adding more Pt/Ti nanosandwiched layers.

3.2 Individualised Chemotherapy Study with Implantable Drug Delivery Device on Pancreatic Tumor Cells

3.2.1 Experimental Setups

The developed implantable drug delivery device was tested with pancreatic tumor cells (Panc-1 and MIA PaCa-2 bought from ATCC, USA) for investigating its treatment effects when used for programmed chemotherapy. *In vitro* tumor cell colonies were being used as the test models. The implantable drug delivery devices were loaded with Doxorubicin (Dox) hydrochloride solution (1 mg/ml, Sigma-Aldrich, Singapore) and then placed close to the cultured colonies (**Figure 3.16a**). Controlled drug deliveries were achieved by setting the powering timings. The chemotherapy effects were evaluated by microscopy and fluorescent

Figure 3.16 **a.** Experimental setups of individualised chemotherapy. **b.** Cultured tumor cell colonies under microscopy. Scale bar represents 200 μ m.

imaging under an inverted microscope (Nikon Eclipse Ti-U). Images were analysed with image processing software (NIS Element and ImageJ).

3.2.2 Cell Culture

Panc-1 cells were cultured with Dulbecco's modified eagle's medium (DMEM, Hyclone), supplemented with 10% fetal bovine serum (FBS, Hyclone), penicillin (100µg/ml) and streptomycin (100µg/ml). MiaPaCa-2 cells were cultured in DMEM with FBS (10%), horse serum (2.5%), penicillin (100µg/ml) and streptomycin. Cell cultured was maintained in the incubator at 37°C, 5% CO₂ concentration and in a humidified atmosphere (The relative humidity is 95%). A two-layer soft agar system was created in Petri-dishes. When tumor cells growth 2×10^3 concentration, cells were treated with trypsin and re-suspended in medium (1 ml per 3.5cm dish) added with low-melting agarose (0.3%). On top of them a solidified medium (1.5 ml per 3.5cm dish) containing agarose (0.6%) was layered. Colonies were incubated for 7 days till they reached certain sizes. In our experiment, only the colonies with at least 50µm in diameter were counted. Colonies were imaged by using the inverted Nikon microscope (**Figure 3.16b**).

3.2.3 Drug Delivery

The drug delivery device was connected to a 9V battery thus a flow rate of 2.2µl/s was offered (Regarding to Figure 3.8). Drug dosage in each delivery process was carefully controlled by setting the device's working duration, and was further confirmed the amount of delivered Doxorubicin by weighting the Petri dish. Cultured tumor cell colonies were treated under an 8-day chemotherapy scheme with 18µg Dox added in total. Three different treatment programs were designed (**Figure 3.17**). For the program named "Direct", it simulated drug administration from syringes which delivered the total 18µg Dox to tumor cells in Day0. The other two programs named "Program I" and "Program II", demonstrated

Figure 3.17: Illustration of treatment programs during the 8 days of *in vitro* experiments:

Direct: 18µg of Doxorubicin delivery in day 0. Program I: 6µg of Doxorubicin delivery in day 0, day 1 and day 2, Program II: 9µg of Doxorubicin delivery in day 0 and day 3.

different flexible drug delivery patterns. For program I, 6µg of Doxorubicin was delivered separately in day 0, day 1 and day 2. For program II, 9µg of Doxorubicin was delivered in day 0 and day 3. All daily deliveries were conducted at the same time 10:00 AM.

3.2.4 Fluorescent Imaging

Doxorubicin has fluorescent emission peaked at the wavelength of 600nm [124]. We utilized fluorescent imaging to trace the drug transportation in each tumor cell colonies during the chemotherapy. A 470 nm blue light was used as excitation. **Figure 3.18** shows the microscope bright-field (**a.**) and fluorescent images (**b.**) of a colony under “Direct” delivery on Day 1. The fluorescent signal was detected from the colony indicating the drug’s uptake by tumor cells, while the untreated group (**Figure 3.18c**) exhibited total darkness.

3.2.5 Individualised Treatment of Cancer

Figure 3.18: Cultured tumor cell colony: **a.** Bright-field microscopy of with 10X magnification; **b.** Fluorescent microscopy on the same colony. **c.** Fluorescent microscopy of control group with no drug treated. All scale bars represent 20 μ m.

Doxorubicin has been reported in several studies for its effects in inhibiting tumor cell proliferation as it interferes with the cellular nucleus functions [125, 126]. In our experiment, the tumor cell colonies in the control group have shown an obvious size increases during the 8 days period (**Figure 3.19a, b**). While in the treatment group, no significant colonies' size

Figure 3.19: **a.** Bright-field microscopy of tumor cell colonies in Day 0. **b.** Microscopy of untreated tumor cell colonies in Day 7. **c.** Colonies in treatment group in Day 0. **d.**

Colonies after the 8 days' treatment. All scale bars represent 100 μ m.

Figure 3.20: **a.** Amplified image of a tumor cell colony in Day 0. **b.** This colonies after 8 days treatment. **c.** Trypan blue staining on colony after treatment. Scale bars represent 20 μ m.

increasing was observed at the last day of experiment (**Figure 3.19c, d**). This result indicated that Dox chemotherapies on the pancreatic cancer cells have successfully inhibited the colonies' growth.

By analysing the close-up images of treated colony on Day 0 and Day 7 of treatment, the visible darkening as well as losing of smooth cellular boundaries further proved that tumor cells were disintegrated and were no longer alive (**Figure 3.20a, b**). Trypan blue dye staining method was being used to check the viability of the cell colonies. The blue color detected inside cells strongly indicated the breaks of cell membrane that allows trypan blue chromophores to penetrate (**Figure 3.20c**). All results confirmed the treatment effects from the chemotherapies that are conducted by the developed drug delivery device.

On top of the basic evaluation of treatment effects, the individualised treatment of cancer was also studied. We carefully investigated the size changes of each tumor cell colony in order to understand their responses under different drug delivery programs (Direct, Program I and Program II). Two pancreatic cancer cell lines were tested to mimic the treatments on two patients. The result of the colonies size measurements are shown in **Figure 3.21**, expressed as a percentage of the original size over time. First of all, the control groups of Panc-1 and MiaPaCa-2 cells demonstrated that the colonies showed a significant increase

in size after 7 days. MiaPaCa-2 cells showed higher proliferation rate (50% size increase) than Panc-1 (30% size increase) when untreated. For the “Direct” delivery program, colonies from both two cancer cell lines exhibited a fast size decreasing from the first day of treatment and this inhibition maintained till the last day (Day 7). As the simulation of drug injection via syringe, the large dosage of Dox in day 0 has introduced sufficient suppressions on cells’ growth and caused cell’s dying. As for the “Program I” delivery, both cell lines showed resistances to the first-day’s treatment with colonies’ sizes increasing. With more drugs being administrated, inhibitions were eventually achieved and the effects lasted until treatment ceased. It can be noticed that Panc-1 cells required a larger amount of drug (18 μ g of Dox delivered by Day 2) to be inhibited than MiaPaCa-2 cells (12 μ g of Dox delivered by Day 1). Also “Program I” showed less inhibition effect on Panc-1 in Day 7 (0% inhibition) when compared to MiaPaCa-2 (10% inhibition). Lastly, “Program II” delivery showed insufficient therapeutic effects in first-day for both cancer cell lines. Cancer cells experienced a three-day’s growing and finally inhibited by the second dose in Day 3. Similar with former observations, Panc-1 cells grow slower but more easily developed resistance on Dox

Figure 3.21: Tumor cell colonies size measurements during the 8 days treatment. Data was presented with mean values $\pm$ *SD*.

chemotherapy. MiaPaca-2 cells had a faster rate of proliferation, but “Program II” treatment exhibited higher degrees of inhibition ratio in Day 7.

Comparing the programmed dosages with the direct dosage in our treatment, higher inhibition rates were measured from the direct dosage. However, it is worth noting that a large amount of drug administered in a short duration would dramatically increase the risk of side effects. For example, it was reported that large doses of Doxorubicin could induce acute heart disorders [127]. Therefore, the programmed chemo-drug delivery that splits one direct dosage into several delivery sessions could avoid the sharp toxicities on other organs than tumor. It shall be also noted that, certain cancers may quickly build up resistances on the chemotherapy. Thus the separated drug dosages may not be sufficient to inhibit the growth of tumor cells (e.g. Panc-1 cells under programmed drug delivery). To treat the cancer may require multiple drugs working together (This topic will be discussed in the following section 4.2 Individualised Cancer Drug Optimisation with Cell-on-a-Chip Device). Generally speaking, drug delivery with the developed device offers hope for the advanced chemotherapy. Close-tumor localized delivery and flexible treatment of chemo-drugs would be helpful for the avoidance of serious side effects. What’s more, the higher controllability on drug delivery provides viable options for doctors to design a suitable treatment scheme on fitting each patient’s needs. Thus individualised cancer treatment can be easily implemented.

3.2.6 Summary of This Study

A MEMS implantable drug delivery device was designed, fabricated and tested with cancer cells. The device employed electrochemical actuation to achieve controlled delivery flow rates with highly system miniaturization. Efforts have been made to enhance the working reliability of device’s actuator. Localized administration of chemotherapy by the developed device was performed on cultured pancreatic tumor cell colonies. High efficiency

chemo-drug deliveries were accomplished by the device, as cancer inhibition was observed after the drug treatments. Furthermore, individualised treatment can easily be performed based on the device's ability in customizing programmed dosages for each patient, which can also avoid serious side effects from overdose drugs. Cancer from two cell lines required different chemotherapy schemes to be well treated. In general, the presented treatment programs showed higher degrees of inhibition on MiaPaCa-2 cells than Panc-1 cells. Other individualised treatments need to be designed to successfully inhibit Panc-1 cancer. Our next study of the drug delivery device aims at the biocompatibility evaluation of device implantation in animals to confirm its safety and reliability in further clinical tests.

3.3 In Vivo Evaluation of Implantable Drug Delivery Device with Kunming Mice Model

3.3.1 Biocompatibility of Implantable Drug Delivery Device

Confirming the safety of implantable drug delivery devices is commonly the priority during its development to clinical applications [35]. The device's biocompatibility must be guaranteed prior to testing the implantable device clinically. The definition of biocompatibility includes the benignity of the relation between an implant and its biological environment and a proper functionality of the device under implantation settings[35]. For implantable drug delivery devices, biocompatibility is mainly dictated by the materials that be chosen in the construction of the devices. Biocompatibility can be affected by several physical features of devices such as the overall size, shape, and surface area. Also, experimental conditions like the time of implantation or the drug delivery rates are related to biocompatibility[35]. *In vivo* evaluations with animal models are essential to investigate the biocompatibility. Among the *in vivo* evaluation techniques, histology and the blood sample assay are most commonly applied. Histology provides a direct assessment on the implant's influences to surrounding tissues, and the blood assay gives general information on tested animal's health[9, 29, 62]. For a successful example, Langer et al. have presented the first clinical trial study of implanting drug delivery microchips to patients [7]. Devices were implanted in the subcutaneous area of the abdomen close to the waistline. Human parathyroid hormone fragments hPTH(1-34) were stored in the device and selectively delivered to patients for a 5 month period of study. Their histology study has confirmed minimal inflammation on the tissue samples surrounding the implanted device. Their blood assay indicated that all biological values were within normal limits. Also patients gave positive opinions regarding the therapeutic application of using the device.

Biocompatibility was one of the key concerns in the design of our MEMS implantable drug delivery device. The whole body of the device is constructed by PDMS [128] and Polyolefin [129] materials, which have been proven to be biocompatible and safe to use even for long term contact with living tissues. Efforts have been spent to achieve a small thickness of the device to avoid large injuries onto the tissue. The drug delivery device may damage surrounding tissues due to the friction and expansion forces which would be significant when movement is generated [130, 131]. Therefore, adequate softness and smoothness were also taken into consideration in the device fabrication with PDMS.

To comprehensively understand the biocompatibility of our developed device, an *in vivo* evaluation with Kunming mice model was performed. 12 devices were subcutaneously implanted into 12 mice for 28 days. Tissue histology and blood sample assay were utilized to evaluate biocompatibility. Furthermore, the devices working performance after implantation was examined by use the device to deliver adrenaline formulations to the tested animals. Treatment effects were observed through monitoring the blood pressures of the tested Kunming mice.

Figure 3.22: Schematic surgical implantation procedures of our MEMS delivery device.

3.3.2 Device Implantation to Animals

Drug delivery devices were fabricated as was previously mentioned in this dissertation (Section 3.1). *In vivo* subcutaneous implantation is a common approach to examine the overall performance of the drug delivery device and chips [6, 46, 62, 132]. In our study, the developed device was subcutaneously implanted on the back of Kunming mice, and the long cannula was inserted into the animal abdominal cavity for drug delivery purpose. Implantations were conducted on female Kunming mice with a body weight of 50-60 g. Mice were housed as 1 per cage, in a room maintained at $23 \pm 2^{\circ}\text{C}$ and $55\% \pm 15\%$ humidity, in a 12 hour/12 hour light/dark cycle. Sufficient food and water were supplied to mice for their freely uptake. Surgery was conducted at a sterilized operating bench and each device was also sterilized in an autoclave before surgery. Each mouse was given an intraperitoneal (IP) injection of 3% pentobarbital solution of 50 mg/kg as anaesthetic. For minimizing invasive wounds, we designed the implantation surgery to only require 3 small incisions. **Figure 3.22** shows the subcutaneous implantation procedures: **1.** A 2 cm-long incision was made through the dermis at the back of mouse, followed by blunt dissection with hemostat to create a 2 cm² pocket as the space for placing drug reservoir. **2.** Another 1cm-long incision was made at

Figure 3.23: a. Photograph of device implantation subcutaneously to mouse.**b.** fur was shaved off on implantation area.

the abdomen followed by blunt dissection towards the first incision to create a 3 cm path between the two incisions. **3.** In the same position with second incision, a third 0.5 cm-long incision was made on the abdominal wall. **4.** Device was placed in the 2 cm² pocket through the first incision and the long cannula was placed into the 3cm-long path with its tip inserting into abdominal through the incision on the abdominal wall. Eventually all incisions on dermis were sutured. **Figure 3.21a** shows a Kunming mouse after device implantation surgery.

All 12 mice remained alive and healthy after surgery. A 28-day continuous health and behavioural monitoring on the tested mice was performed. No scratching or biting to the implants were observed from the animals (**Figure 3.23b**). Tested mice kept a normal frequency in eating and drinking while they demonstrated the same level of activity compared to the control group. Their quick recoveries from surgeries indicate that the minimally invasive surgery did not cause major adverse impacts to the tested mice. It is also worth to mention that the drug reservoir of the device can be easily positioned when using the subcutaneous implantation method, thus the drug dispersion refilling can be possibly achieved with a syringe (**Figure 3.23a**).

3.3.3 Histology

To understand the tissues' responses to our implanted delivery device, a surgery operation was carried out to remove the device at day 2, 4 and 28 days after the initial implantation. At the same time, the wound healing processes were assessed. Tissues samples surrounding the device and 1 ml blood samples were acquired from each mouse for further examinations. The wound healing assessment showed that the wound healing started to occur near the implantation area on day 2 after the device implantation. New fibrous tissues and blood vessel were seen growing on the surface of the drug reservoir (**Figure 3.24a**). The wound healing also continued subsequently. The observation from day 4 showed the

Figure 3.24: Wound healing assessment: **a.** Photograph of tested device taken at day 2.

Yellow arrows indicate newly formed blood vessels. **b.** Photograph of tested device at day 4. **c.** Photograph of tested device at day 28. Device was fully encapsulated by fibrous tissues. **d.** Photograph of long cannula taken at day 28. Yellow arrow indicates cannula inserting through abdominal cavity wall.

formation of more growing tissues (**Figure 3.24b**). By day 28, the wound healing processes were almost completed at the incision sites. The implanted device was observed to be fully encapsulated by fibrous tissues and blood vessels as shown in **Figure 3.24c**. Similarly, wound healing assessment on the abdomen incision area also showed that the placement of the Polyolefin cannula did not slow down the complete recovery (**Figure 3.24d**). All observations showed no severe inflammations or infections. For a short conclusion, wound healing assessment provides basic evidences to the developed device's biocompatibility to surrounding tissues.

Tissue samples acquired from implantation area were embedded with paraffin and then stained by haematoxylin-eosin (H&E) for detailed analysis under microscope. Imaging of tissue samples at day 2 showed slight abnormal tissue responses with implantation. Submucosal edema and capillary extension were observed on the tissues surrounding the implant (**Figure 3.25a, d**). Also, cell infiltrations were found in the edema area. The cells infiltrations were consisted of monocytes, neutrophils and fibroblasts thus they indicating interstitial inflammation. This inflammation has expanded into skeletal muscle with neutrophils infiltration observed. However, the inflammation was too slight to cause any severe immune responses in the tested animal. At day 4, the edema and capillary extension observed previously have shown to be significantly alleviated through the formation of

Figure 3.25: Microscope images of subcutaneous tissue surrounding implanted device, haematoxylin-eosin (H&E) stain. **a.** Photo taken at Day2 with 10X magnification. Black arrow indicates submucosal edema region. **b.** Day4 with 10X magnification. Black arrows indicate the newly formed capillaries. **c.** Day28 with 10X magnification. Black arrow indicates encapsulating epithelioid histiocytes. **d.** Day2 with 100X magnification. **e.** Day4 with 100X magnification. **f.** Day28 with 100X magnification.

granulated tissue (**Figure 3.25b, e**). Additionally, a number of newly formed blood capillaries were found, which confirmed the results from wound healing assessment. However, an obvious amount of monocytes, neutrophils, fibroblasts infiltration indicated the continuing of inflammation. Fortunately, the inflammations were significantly alleviated with continued implantation. Infiltration of both interstitial inflammation cells and inflammation cells reduced to normal amounts based on the observation at day 28 (**Figure, 3.25c, f**). Based on the histological analysis, the encapsulating tissues on implanted device were determined to be the newly grown layers of epithelioid histiocytes. Overall, the H&E staining analysis study confirmed the normal wound healing processes and showed a benign response from tissues with implantation. No other signs of degeneration, bacterial infection, or malignancy were founded from all the harvested tissues.

3.3.4 Blood Sample Assay

Blood sample assay is another general method to evaluate an animal's health conditions [35]. In our test, blood samples were taken on day 2, 4 and 28 for the mice with device implanted and mice without implants as control. Complete blood tests on each blood marker were performed to monitor immune response, liver function, kidney function and blood protein levels. Tissue histology has shown that no severe infection and adverse immune response generated from the device implantation for 28 days. The conclusion was confirmed again by blood tests. Haemoglobin (Hb), total bilirubin (TBILI), direct bilirubin (DBILI), red blood cell (RBC) count, neutrophils (NE), monocytes (MO), lymphocytes (LY), and white blood cell (WBC) count level were all in safe range as comparing to control. Mice's livers and kidneys functioned normally after device implantation, as shown by unchanged alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (CRE), and uric acid (UA) level. No toxicity was observed after the insertion of the device *in vivo*. Besides the assays

Figure 3.26: Each blood marker test result compared with the control levels. *: Lower blood protein levels on day 2 and day 4 due to mice less food intake, returned normal on day 28. **: Higher AST level on day 2 recovered from day 4. Data was presented with mentioned above, it can be noticed that a slight decrease in the measurements of blood protein levels including total Protein (TPROT), albumin (ALB), blood urea nitrogen (BUN) and triglyceride (TRIG) on day 2 and 4 data (**Figure 3.26***). A possible explanation for the decreases in the levels is the decreased amount of food consumed by the device-implanted mice in their early recovery period. The discomfort generated by the implantation on the first few days can affect an animal's appetite based on our observations and experiences. With wound healing, less pain or itches were felt by the tested mice. Therefore TPROT, ALB, BUN and TRIG levels returned to the normal range in day 28's data. We also noted a level of aspartate aminotransferase (AST) higher than normal on day 2 but not in day 4 and day 28 (**Figure 3.26****). As the marker for acute myocardial infarction, AST fluctuation may be

caused by the stress received during implantation surgery process. Eventually, the AST level has stabilized and maintained at usual range after day 4, thus the implantation in longer time did not generate more long term stresses in the tested mice.

3.3.5 Adrenaline Treatment by Implanted Device

Proper functioning is another aspect of biocompatibility of implantable device. A reliable working performance of the implantable drug delivery device is required under implantation settings. This could be challenging as the implanted device is surrounded by a complex biological environment. Moreover, it is difficult to directly observe the working of device after implantation. For our developed drug delivery device, its working performance was evaluated with the use of adrenaline (epinephrine). Adrenaline is known that it affects the blood pressure level therefore its delivery can be traced by monitoring the animal's blood pressure. Among the 12 devices being implanted, 3 devices were filled with 50 μ l adrenaline. After the implantation surgery, devices were powered by an 8V bias voltage for 25 seconds to deliver the total 50 μ l drug solution to the animal's abdominal cavity. The blood pressure values were measured at 3-minute before/after drug delivery by using a non-invasive blood pressure measuring instrument BP2010A. We designed a control group with another 3 Kunming mice under syringe injection of 50 μ l adrenaline in their abdominal cavity. Their blood press values were also taken for comparison with those of the mice in the experimental group. Both systolic blood pressure (SBP) and diastolic blood pressure were (DBP) were monitored.

The measurements showed that the SBP and DBP of both control (**Figure 3.27a**) and experimental groups (**Figure 3.27b**) demonstrated an identical variation after performing the adrenaline treatment to mice, thus confirming that adrenaline worked effectively when administrated by both syringe injection and implanted device delivery. Therefore, we

Figure 3.27: Mice blood pressure measurements: **a.** Control group under syringe injection of adrenaline **b.** Experimental group under adrenaline delivery from implanted drug delivery device. Data was presented with mean values $\pm$ *SD* (N=3).

confirmed that the drug solutions loaded in device's reservoir have been successfully delivered into the abdominal cavity of the animal. In conclusion, the tested devices demonstrated reliable pumping function underneath the subcutaneous tissues.

A fixed flow rate of 2 μ l/s was set in the drug delivery by biasing the actuator with 8V. This setting leads to a burst release of the loaded drug, in which a specific amount of adrenaline (50 μ l) dispersion to the target site within a short period of time (25 s). The burst release is similar with drug infusion by standard injection with a syringe. As previously mentioned, our developed device has tuneable delivery flow rates, thus it allows one to program desired delivery dosages under specific circumstances. For example, drug diffusion with a low flow rate can be achieved with the function of the device, thus the drug formulation can be delivered for a long period treatment. However, several reports have listed that the therapeutic effects or the toxicity of some drugs could differ significantly under different flow rates[133]. Currently, the relationship between the treatment effects and drug delivery patterns by an implanted device has not been comprehensively studied. Especially

for tumor drug delivery, the drug solutions pumping onto tumor surfaces by implanted devices could work differently from the traditional drug delivery through tumor vascular. We aim to investigate this matter in our further studies (Section 4.3 Tumor Drug Delivery Study with Tumor-on-a-chip Device).

3.3.5 Summary

12 MEMS implantable drug delivery devices were implanted subcutaneously to Kunming mice. The minimal invasive surgery required only 3 small incisions thus minimized the harm done to the mice. Histology and blood assays evidenced the mice's successful recoveries from implantation and their healthy conditions during the test. Three implanted devices were being used to delivery adrenaline formulations to mouse. The blood pressure measurement confirmed the drug effects after turning on the device. The developed MEMS implantable drug delivery device demonstrated high biocompatibility and reliable functioning under the subcutaneously implantation settings.

3.4 Conclusion and Future Aims

3.4.1 Conclusion

To overcome the side effects of chemotherapies due to the low drug delivery efficiency to tumors, a localized treatment technique was proposed. This technique runs by implanting a micro-pump close to cancer sites, allowing direct delivery of chemotherapeutic agents onto tumors. In this chapter, the design, fabrication, and both *in vitro* and *in vivo* testing of a MEMS implantable drug delivery device was presented. The device features a very small size, controllable pumping rate, is biocompatible and reliable within live tissues, making it suitable for the proposed localized treatment. The implantable drug delivery device was tested *in vitro* with cultured pancreatic tumor cells. Successful cancer treatment was demonstrated with significant reduction of colony sizes. Under two treatment programs, 10% and 20% size reduction were achieved compared to a 50% size increasing in the control group. By carefully defining the delivery dosages, the inhibition rates in each treatment session can be controlled. Next, animal experiments were conducted with Kunming mouse models to evaluate the device *in vivo*. Histology and blood assays suggested the device to be highly biocompatible during the 28-day implantation. Finally a proof-of-concept demonstration was performed by using the presented device for the delivery of adrenaline into the mice. We foresee this localized treatment technique by using the implantable devices to provide a viable option for safe and flexible chemotherapies so a much suitable treatment plan for each patient could be performed.

3.4.2 Future Aims

Our future studies aim to comprehensively understand the localized treatment effects of using the implantable drug delivery device to treat actual *in vivo* animal tumors (**Figure**

Figure 3.28: The implantation of drug delivery device to nude mouse with subcutaneous tumor. **a.** Photography. **b.** Fluorescent imaging. Orange circles indicate device implantation area. Orange arrows indicate tumor area. The scale bars represent 10 mm.

3.28). Factors including drug types, dosages, delivery flow rates, or multiple drugs cooperation shall be studied as they may influence the final treatment effects. Besides, the safety of conducting a localized cancer treatment must be assessed to avoid any potential side-effects and risks. The ultimate goal is to test the developed device clinically. Cancers like skin cancer or prostate cancer which are easily reached by the device are highly suitable to be treated with such implantable devices.

To further simplify the device design and fabrication processes, 3D printing technology was considered to be employed into the moulding of this implantable drug delivery device. Generally speaking, the current SU-8 mould that has been patterned using photolithography can be replaced by a 3D printed Polylactic Acid (PLA) mould for PDMS soft-lithography (**Figure 3.29**). By using 3D printing, the fabrication can be completed without cleanroom facilities, thus the cost and time-consuming processing are greatly reduced.

Moreover, 3D printing is a user-friendly technology, with which it would be more convenient to design the device on CAD software. It is worth to mention the biocompatible properties of several 3D printing materials thus making it highly possible to directly print the component parts of the implantable device. This is of great significance in offering a customized device to fit the requirements of each single patient thereby making the individualised treatments possible.

To further improve the device's long term reliability is another aim. Although the using of nanosandwiched structure in fabricating our electrode actuator gives a reliable pumping performance in the *in vitro* and *in vivo* experiment, the device's clinical application would certainly request a more reliable device which could be used for a few months. Efforts shall be spent on increasing the lifetime of our device. Because electrolysis introduces damages onto the electrodes, a deposition of porous nano-film on the metal structures to separate electrolysis reactions has been proposed [29]. Another possible method is to change the Platinum/Titanium to harder materials in fabricating the electrode actuator. For example, silicon nitride has attracted our attention because it is much hardness and it can be strongly

Figure 3.29: 3D printed PLA mould for implantable drug delivery device

bonded on the silicon substrate [134], which could significantly increase the mechanical strength of the electrochemical actuator.

CHAPTER 4 ENGINEERING OF CELL-ON-A-CHIP AND TUMOR-ON-A-CHIP PLATFORM FOR INDIVIDUALISED TREATMENT

Drug testing on live biological models (*in vitro*, *ex vivo*, *in vivo* and clinical) are necessary for the pre-evaluation of when new drugs and the corresponding newly developed modalities are developed for clinical treatment [135]. For example, new anticancer drugs are first tested with *in vitro* cell cultures. Techniques such as cell microscopy and nanoscopy, polymerase chain reaction, cytometry, inhibition assay and migration assay can provide biological information to study a drug's delivery, working mechanism and therapeutic effects. Nominated drug formulations are further evaluated in animal models before clinical trials can be performed. In addition to treatment performance, possible toxicities are also evaluated in animal experiments. Eventually, the developed drug formulations are approved by an administrative body (e.g., the U.S. Food and Drug Administration) for use in patients after dozens of years and millions of dollars are spent in drug testing [136].

In cancer treatment, drug testing for individual patients is discussed for the purpose of developing appropriate and individually customized prescriptions, which allow the optimisation of treatment outcomes and the avoidance of side effects from the use of toxic drugs. However, limitations in medical resources and manpower challenge the feasibility of providing proper cures to individual patients. Individualised treatment can only be possible with sufficient data from drug testing on patient's samples such as tumor cells, which would definitely require a complex procedures and a large amount of resources [137]. There is no mature technique for such an application so far.

Microfluidic drug testing platforms are widely seen as a breakthrough in drug testing techniques [12, 88, 89, 138]. The lab-on-a-chip technology transfers manual operations with bulky instruments into a series of automatic processes in microfluidics system. Processes such as cell culture, drug delivery and optical imaging are performed on a chip or miniaturized mini- or micro- systems, which make drug testing much more convenient and cost effective. Therefore, microfluidics drug testing systems are easily approached by researchers, doctors and patients, which are of great significance to individualised treatment.

Furthermore, a microfluidic drug testing system offers precisely modified models such as a single cell or multicellular spheroid, leading to the generation of single-cell assay [4, 79, 82] and tumor-on-a-chip [17, 87, 93, 139, 140] tests. Tissue and organ simulations on microfluidic devices have especially shown the potential to replace animal models in drug tests, which would allow the costs and time required for drug discovery and treatment validation to be greatly minimised. For anticancer drug discoveries, a large number of novel drugs have been developed in recent years, but very few of them have moved on to clinical trials [141]. This technology is of great significance to overcome the current bottleneck.

In this chapter, we present two platforms for microfluidic drug testing. First, a cell-on-a-chip platform focused on individualised cancer drug screening and testing is discussed. The system features a fully stand-alone electrochemical actuator for microfluidic flow controlling, a cell culture microenvironment maintaining system and a fluorescent microscopy interface to achieve an excellent device for lab-on-a-chip drug testing. As a proof-of-concept, individualised drug screening was demonstrated with the successful treatment of a type of pancreatic cancer (Panc-1) that has shown resistance to Doxorubicin treatment. A two-drug formulation was tested on tumor cells, and the treatment effects are analysed based on the data from growth inhibition and migration inhibition assays on the chip.

Next, a microfluidics tumor-on-a-chip platform is presented. The device makes use of a U-shaped microstructure to trap certain number of flowing tumor cells (MiaPaca-2) and continuously culture them in the microstructure until a uniform 3D multicellular spheroid formed. Specifically, the presented device serves as a platform for the study of drug delivery to tumors. The microfluidics tumor-on-a-chip device perfectly simulates drug perfusion to tumors by cooperation with a drug delivery pump. Through this platform, we obtain a relationship between the infusion flow rates and drug transportations into the tumor that also leads to different efficacies of inhibition on the tumor's growth.

4.1 Engineering of a Cell-on-a-Chip Platform for Individualised Cancer Drug Optimisation

4.1.1 Device Design, Fabrication and Characterization

In chapter 3, an electrochemical actuator was introduced. This actuator features small in sizes, controllable in pumping flow rates and reliable in long term use, and it was utilized here in the cell-on-a-chip platform as a fully integrated fluid actuator (**Figure 4.1a**). Comparing to conventional lab-on-a-chip devices that heavily rely on the off-chip syringe pumps for fluid driven, our platform firstly achieved independent fluids manipulations such as cell culture, drug delivery, cell staining and buffer washing for anticancer drug testing in vitro. First, similar to the processes mentioned in section 3.1, the on-chip electrochemical actuator was fabricated on a glass substrate by means of surface micromachining.

Figure 4.1: **a.** Schematics of the design of the cell-on-a-chip platform. **b.** Photography of the platform after assembly.

Nanosandwiched Pt/Ti design was being applied. Each electrode actuator featured a 3mm X 3mm size, and an extremely small thickness of 3 μ m, which is much smaller than most microfluidics actuators. Secondly, a PDMS layer formatted with cell culture reservoirs, drug/medium/buffer chambers and microchannel networks was patterned by means of soft-lithography on a SU-8 mould. In our prototype, four sets of test unit were integrated in one platform. Each unit contained three drug/medium chambers for delivery and 1 cell reservoir for culturing. Every chamber can be selectively pressurized by turning on the electrochemical actuator thus their contents can be delivered into the cell culture reservoir through the microchannel networks. To avoid cross-talk between electrochemically actuated chambers or even backflow from the cell culture chamber to the drug/medium/buffer chambers, the cell culture chamber is designed at lower level of the device so the drug/medium/buffer solutions can drop into the cell culture chamber by gravity but not the opposite. At the same time, the cell culture chamber breaks the fluids connection of each electrochemically actuated chambers thus the crosstalk is avoided. The cell culture reservoir was optical transparent, therefore cells on the platforms were compatible with most imaging techniques such as microscopy and fluorescent imaging. In our study, the dimensions of cell culture chamber are according to the imaging area of our microscope. Thirdly, the PDMS layer and the glass substrate were bonded by spreading a thin film of PDMS in between as adhesive. Lastly, the platform was packaged with electrical connections for further operation (**Figure 4.1b**). The assembled chip measured 3cm long, 3cm wide and 4mm in thickness. All chambers and reservoirs were filled and refilled through syringes. Waste fluids in cell culture chamber can be removed by placing a small tissue in the outlet. It is worth to mention that the combination of chambers, cell culture reservoirs and microfluidics networks can be easily customized in device fabrication to fit the needs from applications.

The prototyped microfluidics drug test platform was designed with following the idea of individualised medical devices, which design to be used on patients' needs under all settings. In another word, whenever the test should be performed and wherever the targeted patients located at, the platform shall be able to function properly. Therefore, it is necessary to reduce the dependence of professional instruments as well as well-trained personals in conducting the drug testing for individual patient. Efforts have been spent on the independently working of the microfluidics drug testing platform. The use of fully integrated on-chip actuation removed the necessity of additional syringe pumps and tubes in the manipulation of micro-fluids in testing. As discussed in section 2.3, not only the bulky and expensive features for professional syringe pumps, but also the low pumping efficiency have impeded the widely applications of microfluidics drug test platform, in which one pump can only manipulate one channel. For the purpose of automatic operation, our drug test platform was commanded by an open-source programmable Arduino UNO Microcontroller Module to achieve self-controlled working (**Figure 4.2**), which offers great conveniences to conduct an anticancer drug testing by replacing the tedious manual operations with the programmed

Figure 4.2: Photography of the microfluidics drug test platform on microcontroller module. Each LED light indicates the “turn-on” of certain drug/medium chamber.

device's self-operations.

Electrochemical actuation offers a controllable pumping flow rates for fluids manipulations on-chip. For our prototype drug testing platform, the on-chip electrochemical actuator was greatly miniaturized comparing to the same actuator in our implantable drug delivery device, thus its pumping efficiency would be lower. We characterized the actual pumping flow rates under each bias voltage by measuring delivered solutions' over a certain period of time. In the meanwhile, temperature fluctuations on the actuators were monitored with an infrared thermometer (FLUKE 568, Fluke Cooperation). Since all devices for in vitro study must be sterilized, the device characterizations were performed after steam autoclave processes. Characterization results were demonstrated in **Figure 4.3**. For the pumping flow rates, an approximately linear relationship of flow rates versus operating voltages was observed from 6 – 9 V, which shows excellent controllability on fluid driven ranged from 25 nl/s to 65 nl/s. However, at higher voltage than 9 V, a drastic increase in flow rate was observed (**Figure 4.3a**). Temperature characterization was performed next. Results indicated that there was no significant change in temperature when the platform was powering under the 6 – 9 V. Nevertheless, an obvious lifting of temperature of 0.4 °C/s was detected when the voltage increased to 10 V. It suggested that when the bias voltage ranges from 6V to 9 V, the actuation forces were dominated by the electrochemical reactions, which caused more forces to be produced with the addition of electrolysis reactions when the operating voltage increased. At higher bias voltages of 10 V however, Ohmic heating effect on the actuator became significant enough to generate actuation forces, which enhanced the pumping flow rates. However, a large amount of heat accumulating in chambers would make the drugs/mediums delivery unpredictable. What's worse is that the increase of temperature may impact the viability of the cells and the physical property of the anticancer drugs such as

fluorescence, nanostructure and surface properties [142]. Therefore, in our uses of the microfluidics drug testing platform, bias voltages were limited to the maximum of 9 V.

By using the nanosandwiched Pt/Ti multilayer electrode structure, the electrochemical actuators on glass substrate gained a long lifetime and a reliable pumping performance. No metal delamination was observed during the characterizations. PDMS constructed microfluidics structures showed robust with no leakage of liquids occurring during the filling/refilling. Also, our platform remained proper functions under frequent autoclave sterilizations (121°C, 10 minutes) and incubations (37 °C, 5% CO₂ concentration, relative humidity 95%).

4.1.2 Cell Culture

Pancreatic carcinoma cell lines (Panc-1 and MiaPaca-2) were cultured in the cell culture reservoir of the microfluidics drug test platform. Panc-1 cells were cultured with Dulbecco's modified eagle's medium (DMEM, Hyclone), supplemented with 10% fetal

Figure 4.3: Characterization and the linear regression of the integrated on-chip electrochemical actuators utilized in the microfluidics drug test platform (Vertical bars represent standard deviation and number of samples for each point = 4).

Figure 4.4: Microscope images of cell culture models: **a.** Low density cell culturing **b.**

High density cell culturing. Scale bars represent 50μm.

bovine serum (FBS, Hyclone), penicillin (100μg/ml) and streptomycin (100μg/ml). MiaPaCa-2 cells were cultured in DMEM with FBS (10%), horse serum (2.5%), penicillin (100μg/ml) and streptomycin. Cell cultured was maintained in the incubator at 37 °C, 5% CO₂ concentration and in a humidified atmosphere (The relative humidity is 95%). When tumor cells growth 2×10^3 concentration, cells were treated with trypsin and re-suspended in medium (1 ml per 3.5cm dish) added with low-melting agarose (0.3%). 25μl of cell suspended solution was loaded into the cell culture reservoir through syringe. Another 25μl DMEM medium was loaded into the 3 medium chambers. After cells' adhesion to glass, medium solution was replaced every day by the platform automatically. The platform was maintained in the incubator for cell culturing.

Two cell culture models were achieved for anticancer drug testing. The first model provided a low cell density in reservoir thus the total cell number can be easily counted (**Figure 4.4a**). The second model had a high cell density which was suitable for the cells' wound healing/migration assay (**Figure 4.4b**). Cell model with higher density is achieved by longer cell culturing on-chip.

4.1.3 Fluorescent Imaging

Figure 4.5: **a.** Microscopy of Panc-1 under the 4 Dox concentrations. First row shows images taken in brightfield. Second row shows fluorescent images of the same cells. **b.** Microscopy of Panc-1 under the 4 Dox concentrations. First row shows images taken in brightfield. Second row shows fluorescent images of the same cells. **c.** Measurement of the mean relative fluorescent intensity of the two cell lines ($n = 4$, vertical error bars correspond to standard deviation).

After cell culturing, one of the drug/medium chambers in each drug test unit was replaced with 25 μl Doxorubicin. Different concentrations of Dox (5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$) are delivered to both Panc-1 and Mia Paca-2 cells (8 experimental groups). The drug concentrations were determined based on our prior experiment that shows the Dox concentration smaller than 5 $\mu\text{g/ml}$ hardly generated treatment effects while the concentration

larger than 100 μ g/ml could introduce strong toxicities on cells. Each chip with four drug test units was assigned to each experimental group (Sample size N=4 tests). After a 24 hours treatment, cell culture reservoirs were flushed by DMEM medium solutions then cells were imaged under a confocal microscope to monitor the drug uptake by cells. Fluorescent images were processed with the use of software (NIS Element). Generally, a positive correlation was observed for both Panc-1 (**Figure 4.5a**) and Miapaca-2 (**Figure 4.5b**). With higher Dox concentration treated, the relative fluorescent intensity from cells increased. When we compared the relative fluorescent intensity between the two cell lines (**Figure 4.5c**), we observed that although both cell lines are pancreatic carcinoma, they demonstrated different sensitivity to Dox. Especially for Dox50 μ g/ml and Dox100 μ g/ml, Panc-1 had a noticeably higher relative fluorescent intensity than MiaPaca-2, which indicated more drugs were taken by Panc-1 than MiaPaca-2 during the 24 hours treatment. In conclusion, drug's fluorescent imaged from cells suggested the proper functioning of the microfluidics drug testing platform to deliver designed formulations and we confirmed the anticancer drug uptakes by tumor cells. Individual responses to the same treatment were firstly observed from the two cell lines on drug's uptake.

4.1.4 Growth Inhibition Study

In our previous growth inhibition study with Panc-1 and MiaPaca-2 colonies (Section 3.2), Panc-1 cells demonstrated higher resistances to Doxorubicin treatments than MiaPaca-2. To comprehensively understand the treatment effects on the selected pancreatic carcinoma cell lines, Doxorubicin drug testing was performed that make use of our prototyped microfluidics drug testing platform. Growth inhibition study was firstly conducted on low confluence cell culture. Cells were treated with four concentrations of Dox (10 μ g/ml, 20 μ g/ml, 50 μ g/ml and 60 μ g/ml) for 72 hours. Drug formulations were continually perfused

into the cell culture reservoir by using the microcontroller to deliver a 7 V pulse to the electrochemical actuators lasting 2 s every 10 minutes. The chamber with DMEM was activated simultaneously with a 7 V pulse to its electrochemical actuators lasting 2 s every 20 minutes. In control group, cells were maintained in DMEM medium for 72 hours. At each day (0, 24, 48, 72 hour), the total cell number in each reservoir was counted under a microscope. The degree of growth inhibition was determined by the cell counts comparing to the data acquired at 0 hour (Normalized as 1.0).

As for the control group of two cell lines, remarkable population increases were identified during the 72 hours observation (**Figure 4.6a**). Similar with our observation in last experiment (**Figure 3.18**), Miapaca-2 cells demonstrated a higher proliferation rate than Panc-1 cells. At 72 hour, the population of two cell lines became approximately equal due to the limited size of cell culture reservoir. For Dox treatment groups, great differences between two cell lines were seen from the treatment results. MiaPaca-2 cells' growth was greatly inhibited by Dox treatment (**Figure 4.6b**). The lowest Dox concentration (10 μ g/ml) group generated 35% reducing of the total population at 72 hour. Higher degrees of inhibition were achieved when the Dox concentrations were increased. It is interesting to notice that the treatments with 50 μ g/ml and 60 μ g/ml concentrations did not generate obvious differences in the degree of inhibition at 72 hour. Therefore we suggested that for long-term treatments, concentrations above 50 μ g/ml may not produce an added inhibitory effect but the higher concentration is proportional to the risk of overdose.

When the same testing was repeated on Panc-1 cells with the same concentrations of Dox, much less of inhibition effects were shown (**Figure 4.6b**). The lowest Dox concentration (10 μ g/ml) group exhibited an 8% population increasing at 24 hour and failed to reduce the number during the whole test. Although higher Dox concentration groups achieved inhibitions, the degrees were much less comparing to the same treatment on

Figure 4.6: Cell counts versus time. **a.** Control groups.**b.**MiaPaca-2 cell line under the four Dox concentrations.**c.**Panc-1 cell line under the four Dox concentrations (Sample size in each group N = 4, vertical error bars correspond to standard deviation).

MiaPaca-2 cells. For example, 50µg/ml Dox generated only 20% population decreasing on Panc-1 but 70% on MiaPaca-2 at 72 hour. Again, Panc-1's resistances to Dox treatment were observed. In conclusion, Dox treatment could not successfully inhibit the growth of Panc-1 cells due to the individual drug resistance of Panc-1 cells. To achieve sufficient treatment effects on Panc-1, further enhancing the concentrations of medication might be needed but this will put patients into a risk of side effects from the overdose.

4.1.5 Migration Inhibition Study

Figure 4.7: Microscope images of wound healing assays. **a.** MiaPaca-2 cell line under four Dox concentrations and control. **b.** Panc-1 cell line under four Dox concentrations and control.

Chemotactic migration is a component of cancer cell migration, and one of the means by which cancer metastasis[143]. Therefore cancer cell's migration inhibition is another important subject in the anticancer drug testing. Wound healing assay is a standard technique to evaluate the drug effects on migration inhibition[144, 145]. High concentration cell culture models were prepared here and a sterile needle was then inserted into the cell culture reservoir to create a vertical streak on cells. The degree of migration inhibition was presented

as the percentage of wound area at the end of test (48 hours) to its original area (0 hours). Larger wound remained would indicate less cell migrations. Wound area in each group was imaged by a microscope and measured with an image processing software (Image J). In control groups, Measurement of both two cell lines showed a decreasing of wound area with time, finally the wound fully healed at 48 hour (**Figure 4.7a, b**). Then different concentrations of DOX (0.005 $\mu\text{g}/\mu\text{l}$, 0.01 $\mu\text{g}/\mu\text{l}$, 0.05 $\mu\text{g}/\mu\text{l}$ and 0.10 $\mu\text{g}/\mu\text{l}$) were examined on the progress of wound healing.

Under the Dox treatments, Panc-1 cells consistently exhibited a slightly larger wound size as compared to control after 48 Hrs (**Figure 4.7b and 4.8b**). However, it can be observed that an obvious cell migration occurred during the first 24 Hrs under all Dox concentrations (**Figure 4.7b**). In the next 24 Hrs, treatment groups under lower Dox concentrations (0.005 $\mu\text{g}/\mu\text{l}$ and 0.01 $\mu\text{g}/\mu\text{l}$) showed continuously wound healing indicating the failure of migration inhibition. Only higher Dox concentrations (0.05 $\mu\text{g}/\mu\text{l}$ and 0.1 $\mu\text{g}/\mu\text{l}$) showed suppression to the wound healing processes with 84.2% and 96.8% wound size remaining at 48 Hrs. It can be noted that an increasing of wound size over the 0.1 $\mu\text{g}/\mu\text{l}$ concentration group was observed during 24 Hrs to 48 Hrs period, the disruption to migration was severe, cellular adhesion was compromised, causing the cells to break away and spread out from one another (**Figure 4.7b**). In conclusion, only high concentrations of Dox could be efficient in the migration inhibition but it may introduce risks of side effects into treatment.

Unexpectedly, it was observed that MiaPaca-2 cell lines responded resistively to Dox as a treatment for migration inhibition, which was opposed to the growth inhibition studies (**Figure 4.8a**). The cell migration is inhibited when a large DOX concentration of 0.1 $\mu\text{g}/\mu\text{l}$ was used, as seen from the wound size remaining at 88.9% after 48 Hrs (Figure 3D). At lower Dox concentrations of 0.01 $\mu\text{g}/\mu\text{l}$ and 0.05 $\mu\text{g}/\mu\text{l}$, cellular migration was only mildly affected as the wound size decreased to 40.0% and 26.1% respectively after 48 Hrs. For the treatment

Figure 4.8: Wound area measurements. **a.** MiaPaca-2 cell line under four Dox concentrations and control. **b.** Panc-1 cell line under four Dox concentrations and control. (Sample size in each group $N = 4$, vertical error bars correspond to standard deviation).

with $0.005 \mu\text{g}/\mu\text{l}$ of Dox, the wound size was larger than the control population for the first 24 Hrs (62.2% for $0.005 \mu\text{g}/\mu\text{l}$ and 45.8% for control), but at 48 Hrs, both populations had nearly equivalent wound sizes (17.6% for $0.005 \mu\text{g}/\mu\text{l}$ and 16.1% for control). This suggests that $0.005 \mu\text{g}/\mu\text{l}$ of Dox or lower concentrations were definitely insufficient to cause a long term reduction in the migration ability of MiaPaca-2 cancer.

Upon comparing the results from both the migration and growth inhibition assays, it was observed that the same Dox treatment produces vastly different responses with two different cell lines as well as two inhibition assays. Therefore, the individualised treatment must not only be specific per individual, it must also be specific for the intended therapy purpose. In this case, a multifunction treatment is required for both growth inhibition as well

as cell migration inhibition. Especially for Panc-1 cells, Doxorubicin treatment was unsuccessful for both assays.

4.1.6 Individualised Treatment for Panc-1

Panc-1 cells demonstrated a strong resistance to Doxorubicin which lead to the poor results in both growth inhibition and migration inhibition testing. As a proof-of-concept demonstration of individualised medicine, the treatment optimisation for Panc-1 was conducted with the use of our microfluidics drug testing platform. Multiple drug formulations were considered for a combined treatment thereby to gain extra inhibitions and to avoid cancer cells building strong resistances to a single formulation[144]. In our test, the drug candidates contained Dox and Anti Insulin-like Growth Factors (α IGF), which was reported that it also generates anticancer effects to tumor cells [145]. Insulin-like Growth Factors (IGF) are known to protect cells against Reactive Oxidative Species (ROS)[146]. This is antagonistic to the action of Dox which induces cellular damage from the reaction of ROS with cellular components from the reDox activation of Dox[147]. The combination treatment of both Dox and α IGF possibly shuts down the antagonistic function of IGF, thus it promotes the action of Dox by impacting the cell by inducing damage to the cell membrane[148]. A number of drug tests (10 groups, Sample size N=4 in each group) with the combined formulation were performed on Panc-1 in a short period (72 hours). With the automations on microfluidics drug test platform, it was much more convenient to conduct treatment optimisation which may involve a large number of operations.

In each drug test unit, the 3 drug/medium chambers were filled with 25 μ lDox, 25 μ l α IGF and 50 μ lDMEM medium solutions. The same concentrations of Dox with previous tests (0.01 μ g/ μ l, 0.02 μ g/ μ l, 0.05 μ g/ μ l and 0.06 μ g/ μ l) and a fixed concentration of α IGF(0.01 μ g/ μ l) were being used. Growth inhibition assays as well as migration inhibition

assays were performed. In the growth inhibition assays, the combined formulation's treatment with all concentrations of Dox demonstrated a notable increase of therapeutic effects (**Figure 4.9**). Cancer cells' population size in each treatment group exhibited continuous reduction during the 72 hours observation (**Figure 4.9b**). At 72 hour, the degrees of inhibition in each group were significantly higher than the single Dox formulation's treatments (**Figure 4.9a**). The lowest Dox concentration group showed a 30% population decrease with adding α IGF comparing to 0% decreasing without adding α IGF. The 30% population decreasing was even higher than the 20% decreasing achieved by the highest fox concentration formulation without α IGF. Interestingly, the control group treating with $0.01\mu\text{g}/\mu\text{l}$ α IGF only did not show any inhibition effect but a 5% population increasing at the end of test (**Figure 4.9b**). This observation suggested that Panc-1 cells have also built drug resistances to a single α IGF formulation's treatment. Therefore, we conclude that combined formulation offers enhanced growth inhibition effects to Panc-1 cells. Its treatment generated

Figure 4.9: Cell total population counts. **a.** Panc-1 cell line under single Dox formulation of 4 concentrations. **b.** Panc-1 cell line under combined formulation of 4 concentrations (Sample size in each group $N = 4$, vertical error bars correspond to standard deviation).

higher degrees of inhibition than both a single Dox and a single α IGF's treatment.

When Panc-1 cells were treated with Dox without adding α IGF, cell migrations could only be stopped with high Dox concentration formulations ($0.05\mu\text{g}/\mu\text{l}$ and $0.1\mu\text{g}/\mu\text{l}$)(**Figure 4.9b**). Furthermore, the single α IGF formulation did not generate sufficient inhibition effects as 50% of total wound areas were occupied by migrated cells at 48 hour (**Figure 4.10a, c**). Fortunately, the combined formulation also showed a better performance in the migration inhibition assays of Panc-1 cells. For lower Dox concentration ($0.005\mu\text{g}/\mu\text{l}$ and $0.01\mu\text{g}/\mu\text{l}$) with adding α IGF, significant inhibitions on cell's migration were observed as the wound areas remained almost the same with its original sizes (**Figure 4.10a**). Software measured 92% and 100% areas maintained for the two groups (**Figure 4.10c**). For higher Dox concentration ($0.05\mu\text{g}/\mu\text{l}$ and $0.1\mu\text{g}/\mu\text{l}$) with adding α IGF, the migration assay could not be completed for the reason of cell's fast death under the treatment, which has destroyed the cell culture models. It also suggested the combined formulation worked effectively on affecting the cancer cells' health.

In conclusion, through the drug testing with the combined formulation, optimised treatment for Panc-1 was achieved. The individualised treatment for Panc-1 successfully overcame its drug resistances to single Dox and single α IGF treatment. We have found a proper treatment plan with low drug concentrations thus it is possible to avoid the toxic risks of overdosing. Although the individualised treatment optimisation involved large number of tests, our prototyped microfluidics drug test platform offered great convenience for this application.

4.1.7 Summary

A microfluidics drug test platform was developed. Cell culturing, drug delivery and optical imaging were compatible in our platform. The fully integrated electrochemical

Figure 4.10: Migration inhibition assay for Panc-1. **a.** Microscope images of wound healing processes under each treatment groups. **b.** Measurements of wound area for single Dox formulation groups. **c.** Measurements of wound area for combined formulation groups and single αIGF group (Sample size in each group $N = 4$, vertical error bars correspond to standard deviation).

actuator provides stand-alone fluid manipulations without the relying on off-chip instruments, which offers great convenience in performing a large number of testing procedures in the treatment of individual patient. As a proof-of-concept, anticancer drug testing was performed

on pancreatic tumor cells in order to optimise unsuccessful treatment. A Doxorubicin and anti-insulin-like growth factors reagent combined formulation was screened through the on-chip growth inhibition assay and migration inhibition assay for successfully treating Panc-1 cancer, which used to show strong resistances to normal treatment with a single drug formulation (Dox or α IGF). We foresee our platform can be a promising candidate as a drug test tool for both drug researchers in drug discovery as well as clinicians in performing individualised treatment.

4.2 Engineering of a Microfluidics Tumor-on-a-chip Device for Drug Delivery Study

4.2.1 Backgrounds

To date, it is generally known that individualised cancer treatment requires a highly focused delivery profile of drugs to tumor sites [23, 85, 88, 149]. As discussed in Chapter 3, some MEMS research groups have suggested that implantable MEMS drug delivery devices can be employed for local drug delivery therapy of tumors generally detected in early stage or for tumors that are located deeply in tissues [9, 150, 151]. Some studies have even shown that implantable drug delivery devices can be engineered specifically that is based on patient needs for effective individualised therapy [12]. However, there is still a relatively large gap that needs to be filled up before one can use the implantable MEMS device for clinical research applications especially there are very limited studies being reported of employing the devices for patients use. It is worth noting that the drug infusion process initiated by implantable drug delivery devices on the tumors is totally different from the traditional intravenous drug therapy approach [152, 153]. To better understand the treatment effects from the MEMS-device provided drugs, the mechanism of how the drugs interact with tumor cells/tissue must be understood in detail thereby allowing one to design a more holistic MEMS device for effective localized treatment of cancers. Nowadays, the drug therapy results obtained from tumor animal models serve as a gold standard for the pre-evaluation of newly developed drugs before they are translated to clinical testing. However, such large scale of in vivo studies generally require tremendous of financial resources as well as the trained researchers, and thus making such studies less feasible for other research communities to take part [85, 88]. Other significant difficulty related to the testing of implantable devices study is that, the commonly used animal models like mouse, rat and rabbit are extremely size

limited for implanting those devices, which are actually designed for human beings. On the other hand, simple *in vitro* models can be used for drugs testing, but they are mostly cell cultures in petri dishes which do not depict realistically all phenomenon and the mechanism of drugs delivery therapy of tumors[85, 154]. It has been reported that a large portion of newly developed anticancer drugs were found to be less efficient when they were tested in animal models in stark contrast with their performance *in vitro* [85].

Considering the challenges of *in vivo* studies, an inexpensive testing platform is needed to pre-evaluate the drug efficacy of the anticancer drugs before pursuing any *in vivo* studies. This will certainly help the biomedical researchers to avoid using unnecessary small animals, such as mice and rats, for testing [85]. The inexpensive testing platforms are recently encouraged by developed lab-on-a-chip technology, which has enabled one to miniaturize bulky instruments into micro-sized parts and integrate them into a single microfluidic device for high throughput analysis of biological samples. For instance, developed lab-on-a-chip devices are able to perform processes such as cell culture [1], drug delivery [13], polymerase chain reaction [155], cytometry [156] and optical imaging [110]. This allows biomedical research involving complex experimental protocols to be carried out in more convenient and cost-effective manners. More importantly, the integration of micro/nano technologies in microfluidic drug testing systems enable the implementation of precisely modified biological models such as single cells or multicellular groupings, leading to the generation of advanced drug testing techniques such as single-cell assay [4, 79, 82], circulation tumor cell/cluster trapping [137, 157-159] tumor-on-a-chip [17, 87, 93, 139, 140] and organs-on-a-chip [17, 67, 95]. Microfluidics drug testing with modified models are able to offer meaningful results for cancer drug testing, which are proven to be reliable in animal experiments [154].

Multicellular spheroid culture techniques have recently been studied and applied in several cancer researches. Compared to monolayer 2 dimensional (2D) cell cultures, cell

spheroid cultures provide a more approximate simulation of a tumor's *in vivo* microenvironment such as tissue-like structure, cell to cell communication and nutrient/drug gradient [1, 154]. Recently, Albanese et al. have studied the size dependence of nanoparticles (NPs) delivery to multicellular spheroids cultures, and the preliminary results were validated by repeating the NPs treatment on tumors cultured in nude mice [154]. Commonly used methods for spheroid formation include hanging drops, culture of cells on non-adherent surfaces, spinner flask cultures, and NASA rotary cell culture systems [88]. Researchers have recently developed several multicellular spheroids models that make use of lab-on-a-chip technology, named tumor-on-a-chip devices [1, 87, 91-93, 140]. Tumor-on-a-chip technology provides a highly controllable fluidic environment for cell manipulations to be accomplished at micro scale. For example, Wu et al. have reported a high throughput multicellular spheroids culture chip that applies the cells trapping method to achieve self-assembly of multicellular spheroids, which significantly reduced the tumor formation time [1]. Tumor-on-a-chip devices can be made to be compatible with most laboratory instruments, like optical microscope for researchers to evaluate and monitor the growth of multicellular spheroids in real time [154]. Tumor-on-a-chip devices can also be easily integrated with other microfluidic system like drug delivery infusion pump, peristaltic pump which offers highly customizable designs of drug testing and screening in short period of time.

4.2.2 Design and Fabrication

Selective cells capturing and subsequently monitoring their growth under the influence of various biomolecules such as proteins, siRNA, and miRNA in a microfluidic system is an essential technique for developing new drug formulations. The ability to sort, culture, treat and image cells within one unit is important for engineering effective drug screening devices such as single-cell assay [4, 79, 82], tumor-on-a-chip[17, 87, 93, 139, 140]

and organs-on-a-chip[17, 67, 95]. Commonly, surface adhesion [72, 107], electromagnetic force [160, 161], optical force [81, 82], spiral force [137, 156, 162], and microstructures [1, 4, 163] have been employed for capturing cells using a microfluidic chip. Among these methods, cell capturing by microstructures has recently gained tremendous attention due to the simplicity of the fabrication process, the ease of manipulating the microstructure geometry, and its ability to work effectively without any support from external instruments such as lasers, optical lenses, and pumps. By using different shapes and sizes of microstructures, single cell models or multiple cells environments can be created for carrying out experiments such as single tumor cell array [80], inhibition study of tumor cells [92] and the generation of

Figure 4.11: Process flow of microfluidic tumor-on-a-chip device

fabrication.(A)Side view.(B)Top view. A microtrap array chip consists of two components: a SU-8 microtrap array cast on glass substrate using photolithography and a PDMS microfluidic channel prepared by soft lithography. The two components are bonded together to form the complete chip.

tumor spheroids [1] for anticancer drug testing. Moreover, in comparison with other 3D tumor spheroid formation techniques like the hanging-drop method, self-assembled multicellular spheroid in the microstructure requires less time to achieve the 3D tumor spheroids.

Here, we developed a microfluidic tumor-on-a-chip approach which was applied to study drug delivery to tumors. The device utilized “U-shaped” microtraps patterned inside the microfluidic channel to accumulate more than one tumor cells and to continuously culture trapped cells till they form a solid 3D multicellular spheroid (**Figure 4.11a, b**). “U-shaped” microtraps array was made of SU-8 photoresist (Gersteltec Engineering Solutions, Pully, Switzerland) and patterned by photolithography. SU-8 photoresist was spin coated onto the glass slide at 2000 rpm for 70 s to achieve 40µm in thickness, followed by a pre-exposure bake (60°C for 10 min then 95°C for 20 min). Each “U-shaped” micro-structure array was generated by an ultraviolet exposure (35 W, 25 s, hard-contact). After a post-exposure baking (95°C, 40 min), the developing of photoresist was performed to release the SU-8 structure (**Figure 4.11a**). On top the SU-8 microtraps, a microfluidic channel layer was built with soft lithography method, which makes of Polydimethylsiloxane (PDMS, 10:1 base to curing agent ratio, Dow Corning, Michigan, USA). The master copy (mould) for PDMS channel was also fabricated with SU-8 by the same photolithography process mentioned above. PDMS was poured onto the SU-8 mould and then degassed in a vacuum oven. The sample was then cured at 120 °C for 20 min. Then microfluidic channel was peeled off from the mould and bonded to the glass slide with its “U-shaped” trapping array (**Figure 4.11b, c**). On each device, 20×10 “U-shaped” micro-structure array was achieved and the sizes of the “U-shaped” inner cavities were designed for forming tumors with variety numbers of cells. The whole device was fabricated by optical transparent materials to enable subsequent microscopy imaging analysis on tumors.

Figure 4.12: Illustration of cell capturing by microtrap array. **(A)** An overall sketch. Blue arrows represent the direction of the flow of cell suspension. Pink spheres represent cells. **(B)** A side view of a microtrap when cell suspension is introduced. The 3 μm gap allows fluid to pass through but it is not large enough for cells to flow through. Thus, cells are retained in the microtraps. **(C)** Illustration of a fully loaded microtrap. Graphs are not drawn to scale.

The height of the PDMS microfluidic channel was designed to be 3 μm higher than that of the microtrap array. This 3 μm gap allows the fluid to pass but not flowing cells (**Figure 4.12A, B**). As a result, cells entering the microtrap due to the flow would be retained there. However, loading cells with high flow rates may lead to the leak of captured cells through the gap. In this study, we observed substantial cell leakage with loading flow rates exceeding 1000 $\mu\text{l}/\text{min}$. We wish to highlight that the prepared devices were fabricated using optically transparent materials such as glass, SU-8 and PDMS and thereby allowing one to use optical imaging techniques for real-time monitoring of cells growth under various external stressors such as drugs and siRNA molecules.

Figure 4.13: Challenges in preparation of microtrap array. **(A)** A bright field image of microtrap array produced after wet etching in SU-8 developer. The patterns are not fully developed due to the residual SU-8 surrounding the microtraps. **(B)** The same microtrap array after oxygen plasma treatment. The patterns are fully developed as SU-8 residues are removed. **(C)** and **(D)** Images of microtraps after being rinsed with PBS buffer at flow rates of 500 $\mu\text{l}/\text{min}$ and 3000 $\mu\text{l}/\text{min}$, respectively. For low flow rate, air bubbles (white arrows) are trapped in microtrap cavity and thus diminish the cell capturing ability. For high flow rate, air bubbles are washed away and the microtraps are completely wetted. **(E)** and **(F)** Simplified illustration of complete wetting where liquid wet a rough surface perfectly, and composite wetting where the liquid cannot fill in the roughness grooves of a surface. All scale bars, 80 μm .

During chip fabrication, after photolithography, the glass substrates were immersed into SU-8 developer to etch off unwanted parts and release patterns. However, incomplete etching in this development process resulted in residual SU-8 surrounding the microstructures

(**Figure 4.13A**). This underdevelopment is attributed to composite wetting phenomenon, which occurs during the etching process.[122] In composite wetting, the liquid cannot fill in the rough grooves of a surface, resulting in a composite of liquid and gas at the liquid-solid interface (**Figure 4.13F**). Due to this imperfect contact between liquid and solid, the etching solution cannot thoroughly fill the cavities of the microtraps, leading to incomplete etching of the unwanted parts. To overcome this issue, the samples were cleaned by oxygen plasma treatment, by which the undesired SU-8 residue could be removed (**Figure 4.13B**). Additionally, composite wetting can dramatically diminish the capturing capability of the microtrap array. As a result of the composite interface, air bubbles tend to form at the interior of microtraps when cell suspension is flowing through the chips (**Figure 4.13C**). In order to prevent this, PBS was streamed at very high flow rate through the chip to completely wet the microtraps before loading the cells (**Figure 4.13D**).

4.2.3 Multicellular Spheroid formation

The number of cells being captured within the microtrap plays a critical role in the preparation of *in vitro* cancer models for drug testing and evaluation analysis [85]. For example, several approaches have been used for isolating circulating tumor cells, facilitating early cancer detection from blood samples in the near future.[157, 158, 163]By manipulating the sizes of microtrap, it allows one to precisely capture the desired number of cells and thereby obtain different types of *in vitro* cancer model. Three sizes of U-shaped microtraps (Microtrap A, Microtrap B and Microtrap C) were fabricated in this work (**Figure 4.14A-C**). Based on our findings (**Figure 4.14D-G**), Microtrap A can be used to capture single tumor cell, allowing one to obtain a homogeneous single-cell array. Microtrap B and C can capture 3-5 tumor cells and 12-15 tumor cells respectively within the cavity of microtrap. We envision that Microtrap B can be applied for capturing of circulating tumor cell clusters [157],

Figure 4.14: Tumor cell capturing using three microtraps of different size. (A) to (C) SEM images of Microtrap A, Microtrap B and Microtrap C, respectively. (D) to (F) Bright field images of Microtrap A, Microtrap B and Microtrap C with captured tumor cells. (G) Summary of the number of cells captured in three different microtraps.

Microtrap A with inner dimension of $15\mu\text{m} \times 15\mu\text{m}$ can capture single cell. Microtrap B ($30\mu\text{m} \times 30\mu\text{m}$) and Microtrap C ($40\mu\text{m} \times 60\mu\text{m}$) can capture 3-5 and 12-15 cells respectively. Data is presented as mean values with standard deviation (n=20).

while Microtrap C is useful for culturing tumor spheroids in understanding the passive uptake of nano-formulations of drugs.[154]

The biocompatibility of a microfluidic chip is crucial in culturing healthy cells for further experimental usages such as imaging, dynamic tracking of biomolecules within a single cell, and drug testing. Our microtrap array chips are made of SU-8 photoresist and PMDS material on glass substrate. All these materials are known to be biocompatible. For example, Yu et al. employed SU-8 geometric barriers on glass substrate to trap LCC6/Her-2 breast tumor cells and these cells were further cultured for 4 days to generate tumor spheroids [164]. To evaluate the biocompatibility of our devices, the captured cells were cultured on the chips for 96 hours and the cells viability was monitored every 24 hours. Our results showed that the proliferation of tumor cells can be clearly observed after 48 hours of culturing (**Figure 4.15A and B**). As time progressed, a large fraction of cells were observed to congregate around the microtrap structure (**Figure 4.15C**). After 96 hours of culturing, the cells grew into spheroid-like tumor cell spheroids bonded to the microtrap (**Figure 4.15D and F**). Overall, the fabricated chip is suitable to be used for capturing and culturing of cells for more than 96 hours.

4.2.4. Inhibition of Tumor Growth

In this section, we demonstrated the use of the engineered microtrap array chip to monitor the inhibition rate of pancreatic cancer cells (MIA PaCa-2) treated with Doxorubicin (Dox), a commonly used anticancer drug formulation in clinics. The effects of different perfusion parameters of Dox were investigated for optimizing the inhibition effect on tumor cells captured within the microtrap. Two perfusion schemes were studied in this work. In the perfusion scheme 1, Dox suspension was pumped into the device at a flow rate of 100 $\mu\text{l}/\text{hour}$ for an hour. In perfusion scheme 2, the process was repeated using a flow rate of 10 $\mu\text{l}/\text{hour}$ for 10 hours. The overall size of the tumor cell spheroids bonded to each microtrap was monitored after Dox suspension treatment. From our observations, the size of the tumor cell

Figure 4.15: MIA PaCa-2 cells are cultured on the microtrap array chip for 96 hours. (A) to (D) Bright field images of cells within the microtraps after 0, 48, 72 and 96 hours of culturing, respectively. Cell proliferation can be clearly observed after 48 hours. (E) and (F) Close-up images of cells in a single microtrap after 0 and 96 hours of culturing. Cells congregate around the microtrap structure, forming a spheroid-like tumor cell clusters. All scale bars, 30 μm.

4.2.4 Drug Delivery by Infusion Pump and Fluorescent Microscopy Analysis

spheroids in control groups had increased substantially after 48 hours of incubation, with a tumor cell spheroid viability of 93.3% (Figure 4.16). In perfusion scheme 2, the growth of tumor cells were significantly inhibited. A tumor cell spheroid viability of 6.7% was

Figure 4.16: Study on the impact of two Dox perfusion schemes on inhibiting the growth of tumor cells within microtraps. 9 Microtrap C chips with captured cells were divided into three groups. For the two experimental groups, Dox perfusion was performed according to schemes 1 and 2, followed by 48 hours of incubation. For control group, cells were culture for 48 hours without Dox treatment. Tumor cell spheroid viability is calculated as $\frac{N_2}{N_1}$, where N_1 is the number of tumor cell spheroids contained within the microtraps before Dox treatment, and N_2 is the number of tumor cell spheroids which have outgrown to the microtraps 48 hours after Dox treatment. Data is presented as mean values with standard deviation (n=3).

determined, demonstrating that this designed perfusion parameters were effective for inhibiting the growth of MIA PaCa-2cancer cells. On the other hand, in perfusion scheme 1, the growth inhibition effect was observed to be weaker as compared to perfusion scheme 2. A tumor cell spheroid viability of 81.7% was observed in this case.

To further probe the differences between the two perfusion schemes, Rhodamine 6G organic dye was used to monitor their distribution in the tumor cells (**Figure 4.17**). When applied with a flow rate of 100 $\mu\text{l/hour}$ for an hour, the average fluorescence intensity from the stained tumor cell spheroids decreased by at least 75% at 48 hours after treatment as compared to the initial value measured at 30 minutes after treatment (**Figure 4.17E**). When a flow rate of 10 $\mu\text{l/hour}$ was employed for 10 hours, a 15-20% decrease in the fluorescence

Figure 4.17: Study on the distribution of Rhodamine 6G in the tumor cells over time.

Fluorescent images were taken and fluorescence intensity was measured at 12 hour intervals. Fluorescent images of tumor cells undergoing Rhodamine 6G treatment at a flow rate of 100 $\mu\text{l/h}$ for 1 hour taken at (A) 30 minutes and (B) 48 hours after the treatment. Fluorescent images of tumor cells undergoing treatment at a flow rate of 10 $\mu\text{l/h}$ for 10 hours taken at (C) 30 minutes and (D) 48 hours after the treatment. (E) Normalized fluorescence intensity of tumor cells versus time. Normalized fluorescence intensity is calculated as the ratio of the fluorescence intensity at 12, 24, 36 and 48 hour over the initial value measured at 30 minutes upon completion of Rhodamine 6G treatments. Data is presented as mean values with standard deviation ($n=10$). All scale bars, 20 μm .

intensity was observed (**Figure 4.17E**). Based on this finding, we speculate that a short therapeutic period of Dox treatment (perfusion scheme 1 in above section) was insufficient to initiate an effective drug delivery therapy *in vitro*. On the contrary, by extending the therapeutic period while maintaining the same drug dosage, a more potent effect was detected from Dox delivery therapy.

Based on the test results from fluorescent imaging, we suggest that the possible reason that leads to the fail of Dox treatment could be the poor drug transportation into spheroids under fast infusion. It is worth to mention that, a poor drug delivery process could also result to serious side effects. In our preliminary *in vivo* experiment, fast infusion of quantum dots (CdTe QDs) solution to tumor was conducted by an implantable drug delivery device. However, the followed fluorescent imaging showed that delivered QDs failed to penetrate into tumor but they spread in the mouse's abdominal cavity, which caused serious toxicity to test animals (**Figure 4.18**). In conclusion, the slow infusion pattern may be more suitable for our future implanted anti-cancer drug studies for its superior performance in our on-chip tumor inhibition assay.

In this work, we have discovered that the captured and cultured tumor cell within the microfluidic device has a different physiological behaviour as compared to culturing tumor cells in a petri dish. When the two designed Rhodamine 6G perfusion schemes were performed on petri dish-cultured tumor cells, no significant fluorescence intensity difference was observed between the two perfusion rates. Several studies have reported that trapped tumor cells within the microstructure could potentially form a single spheroid and can be treated as an *in vivo* tumor model for drug pharmacokinetics studies.[1, 87, 92] Many works have reported that tumor spheroid models are typically much more resistant to multiple drugs therapy as compared to petri dish-cultured tumor cells since the fundamental physiological behaviours of these two models are different in the aspects of cell-to-cell interaction,

biomolecules uptake, protein excretion, and gene expression [85, 88]. We therefore foresee that there is a need in engineering a microfluidic device that has the capabilities to selectively capture and culture desired type and number of cells for subsequent generation and study of spheroids under the exposure of various drug formulations.

4.2.6 Summary

In summary, arrays of single cells, 3-5 cell clusters and 12-15 cell spheroids were

Figure 4.18: *In vivo* fluorescent imaging on nude mouse after it had been implanted with a quantum dots (QDs) infusion. *In vivo* delivery of QDs solution in high flow rate showed poor delivery efficiency into tumor but high risks from drug diffusing over other areas (e.g. brain). Miapaca-2 cancer cells were seeded subcutaneously near the leg. The implantable drug delivery device was implanted subcutaneously on the back of the nude mouse, for $N = 3$ mice.

achieved using our fabricated microtrap array chips. MIA PaCa-2 cells were cultured in the chip for more than 96 hours with notable proliferation. An *in vitro* tumor cells growth inhibition study was performed using the device, leading to the following observations: (a) Dox delivery therapy at flow rate of 10 $\mu\text{l}/\text{hour}$ for 10 hours showed higher inhibition effect on the growth of tumor cells as compared to a flow rate of 100 $\mu\text{l}/\text{hour}$ for 1 hour. (b) Fluorescence of tumor cells stained with Rhodamine 6G faded faster when the dye treatment was performed at 100 $\mu\text{l}/\text{hour}$ for 1 hour as compared to 10 $\mu\text{l}/\text{hour}$ for 10 hours. We anticipate that our microtrap array chips will be useful in single-cell assay, tumor-on-a-chip culturing and anticancer drug screening.

4.2.7 Perspective

The tumor-on-a-chip device and oncometry method could open up new avenues for future anti-cancer drug applications. We foresee that further development of our tumor-on-a-chip device would leverage on the strengths of many other lab-on-a-chip techniques to provide a more powerful and easy-to-use tool for drug developers. Indeed, the electrochemical actuator used in the cell-on-a-chip device can be integrated with the tumor-on-a-chip device as an on-chip pump which offers standalone fluid manipulations. In other words, the tumor-on-a-chip device can be combined with the cell-on-a-chip device by changing the cell chamber to a tumor chamber thus anticancer drug testing can automatically perform on these tumor spheroids. However, it is worth to mention that the current using of the tumor-on-a-chip device remains few manual operations such as cell re-suspension and flow. More efforts must be spent for the further automation of such device. On the other hand, approaches for microscope-on-chip have been demonstrated showing great potential towards techniques without relying on an external microscope [107, 109, 165, 166]. Moreover, the acquired images could be processed by image processing software on PCs [167] or smartphones [110];

thus, automatic testing/analysing of anti-cancer drugs would be possible. With those improvements, the eventually clinical applications of tumor-on-a-chip devices can be possible, which would greatly benefit to the planning of a suitable treatment tailored for each patient.

4.3 Conclusion and Future Aims

4.3.1 Conclusion

In this chapter, two microfluidic drug testing platforms were developed for our proposed individualised tests. The first cell-on-a-chip drug test platform utilized fully integrated electrochemical actuators to achieve automatic cell culturing, drug delivery and buffer/medium washing, which offered great convenience and ease-of-use in conducting multiple anticancer drug tests on live tumor cells. Using this platform, we carefully investigated the Dox treatment on two pancreatic tumor cell lines through the growth inhibition assay and the migration inhibition assay. We earlier found that the Panc-1 cells developed a strong resistance to the treatment therefore another Dox and Anti Insulin-like Growth Factors (α IGF) combined formulation was selected to further optimise the treatment for Panc-1. Successful treatment with inhibitions on both the growth and migration of Panc-1 cells was obtained using the combined formulation.

Furthermore, we introduced a tumor-on-a-chip device. This device provided *in vitro* 3D multicellular group models for the simulation of multicellular spheroids, which are more close approximations of real solid tumor than monolayer cell culture. Using this device, we studied multicellular spheroid's responses to drug infusion treatment with a delivery device. We observed that the drug infusion patterns were crucial to the treatment effects. Drug infusion with slow flow rates would lead to higher efficacy in the drug transportation to spheroids thus it would generate higher degrees of inhibition on spheroid's growth. Ease-of-use, high throughput and low cost are advantages of microfluidics drug test devices, which fit the requirements in the proposed individualised drug test.

4.3.2 Future Aims

Our following studies aim on validating the significance of the developed drug test devices. Drug tests conducted on chip shall be examined again with standard procedures including animal experiment and clinical trials in the future. Another focus is to use samples directly from patient for developing individualised treatments, for anticancer drug tests, techniques for detection and enrichment of viable circulating tumor cells are considered as the source of viable cell samples for our microfluidics drug testing devices[137, 158].

In our tumor-on-a-chip device, we utilized microstructures to trap multiple tumor cells for testing. Additionally this technique can be easily modified to be applied in single cell studies. By miniaturizing the microstructure, single cells can be isolated and located in the microtraps array (**Figure 4.11a**). Furthermore, by tuning the size of microstructure, it is possible to screen certain type of cells from others. For our proposed study, the techniques to capture circulating tumor cells from blood stream could be merged with the microfluidics drug testing platform. Tumor cells trapped directly from a patient's blood are suitable to be treated, imaged and counted on chip and thus provide meaningful information for further cancer diagnosis, monitoring and drug screening of individual patient.

Besides tumors, other organ models can be cultured in microfluidic devices to closely simulate their behaviour in the human body. This idea has been developed recently and generated the organs-on-a-chip devices. The next level of complexity for this technology focuses on developing more complex multiple-organ systems including tumors (**Figure 4.19**). Interactions between each on-chip organs are realized by microfluidic networks which simulate the human circulation system. We foresee that such multiple-organ systems would be able to providing a promising image to future individualised biomedical applications, especially for clinical anti-cancer drug testing which evaluates side effects on other normal organs than tumors.

Figure 4.19: An illustration of multiple liver/tumor co-culture system with micro-channel network for circulating medium.

Lab-on-a-chip applications are versatile platforms, which can perform many essential functions. To further simplify the drug testing platform's design and fabrication processes, 3D printing technology was considered to be employed into the moulding process. Similar with the case of the implantable drug delivery device (**Figure 3.26**), the SU-8 photolithography mould can be replaced by a 3D printed Polylactic Acid (PLA) mould for subsequent PDMS soft-lithography of the microfluidics structures. By using 3D printing the fabrication no longer needs cleanroom facilities thus the needed cost and time are greatly reduced. Moreover, 3D printing is a user-friendly technology which is more convenient to use even by non-specialists who can design the device on CAD software. This is of great significance as it is important step towards offering customized platforms for specific cases. Thereby, lab-on-a-chip applications can be widely used in individualised medicine.

CHAPTER 5 CONCLUSIONS

Individualised medicine, which aims to comprehensively optimise diagnostic and therapeutic strategies for the specific treatment of individual patients, has been greatly advanced by the recent development of novel microfluidic devices based on MEMS technology. Microfluidic enhances the precision with which fluids, cells and nano/micro-objects can be manipulated and at the same time offers a solution for the achievement of complex biochemical applications with a miniaturised device or platform. Plenty of new applications in individualised medicine, such as real-time diagnosis, pre-evaluation of drug effects, and controlled drug delivery for a single patient, have been developed that demonstrate a number of advantages in overcoming the shortcomings of conventional medicine. Furthermore, these low cost, easy to use, small microfluidic devices can provide necessary medical services to patients when resources are limited. Medical tests that used to be accomplished only in a hospital or laboratory with tedious technical procedures can now be conducted for each patient wherever and whenever he or she needs it.

This dissertation presents two approaches is using microfluidic technology to design, mould, fabricate and test microfluidic devices for individualised medicine. The first approach focuses on an implantable drug delivery device for localized administration of chemotherapy. The device is designed for efficient and controllable delivery of chemotherapeutic drugs to tumors to allow the reduction of side effects during treatment. Soft lithography, photolithography and surface micromachining were involved in the microfabrication of the device. The completed device is soft, flexible and small, which reduces the risks of implantation. Electrochemical actuation provides pumping forces for the devices. The control of drug therapy was achieved by using the linear relationship between pumping efficiency and bias voltage. Efforts have been made to improve the reliability of electrochemical

actuation. A robust electrode structure of nanosandwiched metal layers was introduced to overcome metal delamination in actuation, which greatly increased the total lifetime of the drug delivery device for long-term treatment.

The prototype device was first tested on pancreatic cancer cells. The device's anticancer performance was demonstrated by a significant reduction in the sizes of the tumor cell colonies. Size decreases of 10% and 20% were achieved, in comparison with a 50% size increase in the control group. The device offers the unique feature of formulating a specially designed treatment plan on a patient-by-patient basis. Under the programmed drug delivery dosage, individualised treatment effects were observed on different types of tumor cells.

Next, animal experiments were performed to evaluate the device's reliability and safety for clinical use. The safety of the device implantation was confirmed by the 100% survival rate of the tested animals. Tissue histology and blood assay indicated the good biocompatibility of the developed drug delivery device. The working performance of the device was also found to be reliable by completing drug delivery to the animals. With their *in vivo* evaluations, we gained great confidence for future clinical testing of the device for cancer treatment.

For the second microfluidics approach, we constructed a prototype cell-on-a-chip platform for cancer drug screening and testing in two versions. The cell-on-a-chip drug testing platform utilised fully integrated electrochemical actuators to achieve automatic cell culturing, drug delivery and buffer/medium washing, which for the first time removed the reliance on syringe pumps or manual operations in a drug testing with live tumor cells. The platform thus offers great convenience in conducting biochemical tests on live cells. Using this device, we carefully investigated Dox chemotherapy effects on pancreatic tumor cells in forms of growth inhibition and migration inhibition. We detected Panc-1 cells that

demonstrated strong resistance to the treatment and selected another combined formulation of Dox and anti-insulin-like growth factor to further optimise the treatment for Panc-1. Successful treatment, with inhibition of both the growth and migration of Panc-1 cells, was observed with the combined formulation. The proof-of-concept study showed the potential to improve cancer treatment on an individual patient by drug testing using a microfluidic device.

In the last section, we introduced a tumor-on-a-chip device for drug testing. This device provided *in vitro* multicellular spheroid cultures that were closely approximate a real solid tumor than monolayer cell cultures; thus, it could offer more meaningful information for the evaluation of drug therapy. With this device, we studied the tumor's responses to treatment with a drug delivery pump. We observed that the drug infusion patterns were crucial to the treatment effects. Slow flow rates of drug infusion led to greater efficacy in drug transportation into tumors and thus generated greater degrees of inhibition of the tumor's growth during treatment.

Microfluidic drug testing devices have showed the advantages of ease of use, automatic operation, high throughput and low cost, which fit the requirements of the our proposed individualised applications.

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LIST OF ABBREVIATIONS

ALT	Alanine Aminotransferase
ALB	Albumin
ALP	Alkaline Phosphatase
AST	Aspartate aminotransferase
BUN	Blood Urea Nitrogen
CO ₂	Carbon Dioxide
CTCs	Circulating Tumor Cells
CAD	Computer Aid Design
CRE	Creatinine
DI Water	Deionized Water
DBP	Diastolic blood pressure were
DBILI	Direct Bilirubin
Dox	Doxorubicin
EDS	Energy Dispersive Spectroscopy
FDA	Food and Drug Administration
Hb	Haemoglobin
ITO	Indium Tin Oxide
IGF	Insulin-like Growth Factors
LOC	Lab-On-A-Chip
LED	Light-Emitting Diode
LY	Lymphocytes
MEMS	Micro-Electro-Mechanical System
MTTF	Mean Time to Failure
FBB	Microfluidics Breadboard
MO	Monocytes
NPs	nanoparticles

NE	Neutrophils
O ₂	Oxygen Gas
PDMS	Poly(Dimethylsiloxane)
PLGA	Poly-(Lactic-Co-Glycolic Acid)
PLA	Polylactic Acid
PCR	Polymerase Chain Reaction
ROS	Reactive Oxidative Species
RBC	Red Blood Cell
SEM	Scanning Electron Microscopy
SiO ₂	Silicon Dioxide
SBP	Systolic blood pressure
3D	Three Dimensional
TiO ₂	Titanium Dioxide
TBILI	Total Bilirubin
TPROT	Total Protein
TRIG	Triglyceride
UA	Uric Acid
WBC	White Blood Cell

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