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Current progress in micro/nanofluidic chips and applications in cancer research and therapy

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Abstract. Micro/nanofluidic chips have set a new stage for cancer research and therapy, revolutionising the way we detect, diagnose, and treat this formidable disease. This paper provides an overview of the basic properties of micro/nanofluidic chips and current progress in the utilization of micro/nanofluidic chips in the realm of cancer. Their application in DNA and protein analysis, their role in cancer modelling and drug testing, and their innovative use in drug-eluting devices for cancer immunotherapy are discussed. The advantages and limitations of these technologies are evaluated, shedding light on the challenges and opportunities. Having the potential for earlier and more accurate diagnosis, novel therapy methods with better outcomes and less side-effects, more advancement and further breakthroughs can be anticipated in tackling tumours with micro/nanofluidic chips. In future development, it is suggested that combinations of such chips and various other emerging technologies can be attempted and explored for inventing more innovative and functional micro/nanofluidic devices, making a difference in the field of cancer research and therapy. Simultaneously, more improvement such as enhancing reproducibility and affordability is also necessarily required to realise the clinical trial, mass production, commercialisation of these chips.

Keywords: Nanotechnology, micro/nanofluidic chip, genomics, tumour modelling, immunotherapy

1. Introduction

Cancer diagnosis and treatment has been a challenging topic for decades. Annually, numerous lives are claimed by malignant tumours. The current treatment process also brings pain and suffering to countless patients. To overcome this disease which has a significant death rate worldwide, there is the urge to develop new methods to study it deeper and to defeat it. To reduce the limitations and side effects in traditional cancer solutions, advancement in microtechnology and nanotechnology in recent years have brought new possibilities towards cancer research and therapy [1, 2].

The extremely tiny dimension and the large surface-area-to-volume ratio of nanostructures and nanomaterials equip them with numerous unique physical and chemical properties and thus advantages that can hardly be replaced by conventional material, such as their great conductivity, binding and absorption ability, biocompatibility, etc [1]. These properties allow nanomaterials to bring many revolutionary solutions for cancer. For instance, gold nanoparticles have the ability to efficiently carry molecular drugs and travel through human bodies to bind with targeted tumour cells [3]. Not only in

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drug delivery, nanotechnology is also widely used in other applications for cancer such as image enhancement for cancer diagnosis and molecules isolation for tumour studies [1].

One of the most recent and popular studying focuses on nano-related strategies for cancer can be nanofluidics. The concept of nanofluidic devices first came out in the early 21st century. Due to their unique properties and capabilities such as nano-scales structures, strong confine ability and capillary and so on, they have provided us new methods to handle and manipulate the fluids. They are now greatly progressed to customise and fulfil a wider range of functions. They are greatly helpful in biomedical research as they share a similar size with human body cells and proteins. Seeing its great potential in biochemical and medical fields, many new innovative micro/nanofluidic chips are designed and invented for biomedical purposes. A lot of information about micro/nano-fluidic chips is known based on currently available academic papers and related studies. New devices, micro/nanofluidic chips, have been invented and widely used in oncology research and cancer therapeutic innovations, but there is a lack of systematic papers on the latest development of these chips and their applications in cancer studying.

This paper aims to summarise the current progress of micro/nanofluidic chips in cancer research and therapy, and to analyse and discuss the advantages, challenges, and potentials of the development of micro/nanofluidic chips in this field, providing new insights into future cancer solutions [1,2].

2. Micro/Nanofluidic Overview

2.1. Micro/Nanofluidics

Micro/Nanofluidics is the science of manipulating and controlling fluids at the micro-/nanoscale, typically within channels and structures with dimensions of micro-/nanometres [2]. Fluids confined in such tiny structures can show unique physical and chemical properties, presenting the real activities of single molecules more accurately [4, 5]. This is especially useful in studying the activities of tiny molecules in human cells such as DNA and proteins which are in the dimension of nanometres or even smaller. Therefore, many scholars tried using this technique to study pathological cell events and it has shown strong potential in cancer research and therapy [1, 2].

2.2. Micro/Nanofluidic chips

A micro/nanofluidic chip offers a miniature yet powerful platform for precise manipulation and analysis of fluids at the micro or nanoscale [5]. The device is equipped with an intricate network of nanometer-sized channels, (or chambers and pores) that enable the controlled flow, confinement, and interaction of minute volumes of fluids and particles [5]. Several reservoirs which can be seen by naked eyes are usually designed to connect with the tiny structures for the convenience of injecting fluids into the chips. There is a wide range of applications of nanofluidic chips, from DNA sequencing and single-cell analysis to drug delivery and the investigation of fundamental biological and chemical processes [5]. By harnessing the unique properties of micro/nanofluidics, researchers are able to use thischips to conduct new research methods in biomedical fields, revolutionizing the approach to scientific exploration and medical therapy.

3. Application of micro/nanofluidic chips

3.1. Cancer studying (DNA analysis)

Using microfluidic systems as a platform to isolate and detect tumour cells has been studied in numerous types of cancers [6]. Nanofluidic system with stronger isolation and confinement ability can be used for more detailed analysis of tinier molecular unit. Different nanofluidic chips are designed for use in predicting different cancers, based on the number and size of enzymes or biomarkers needed for the detection [7, 8]. With the help of linearising single-strand DNA by nanofluidic chips, DNA sequencing - base pairs (A, T, C, G) which denotes higher risk for cancerous development can be detected and counted [8, 9]. This could be useful in genetic or early cancer prediction and future studies in revealing

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the DNA appearance of cancer patients. Information of cancer subtypes can also be obtained and thus more personalised treatment plan can be designed for the patients for more effective treatment outcomes.

3.2. Cancer modelling and drug testing

Micro/nanofluidic chips can be used in tumor modelling for cancer studying and cancer drug testing, replacing using animal bodies as an experimental base. The chip designed with micro and nanochannels inside manage to simulate and surpass the cancer xenograft model in certain cancer and drug studies. In one of the recent studies, the efficacy of oxaliplatin against HCT116 colorectal tumour is tested by placing the cancer spheroids inside a microfluidic chip, with exposure of the spheroids to dynamic, in vivo-like concentrations of oxaliplatin [10]. The chip is designed with two main parts, with a straight microchannel on top to simulate the continuous provision of nutrients and medicines and a well below for placing and culturing a single spheroid [10]. By comparing different properties of the growth and shrinking of the spheroid among chip, xenograft implantation in mice and the control group, it has been shown that the microchannel chip system is able to recapitulate important characteristics and activities of tumours and xenograft models. Such microfluidic chip systems are getting more popular nowadays for cancer modelling and drug testing. This is because these chips not only can functionalise as xenograft implantation without interfering the spheroid growth and changing the drug efficacy, but also can overcome some limitations of other models (eg. 2D cultural models, xenografts models) and provide more drug response information than other methods. Overall, the recent chips manage to simulate the drug efficacy in xenograft models by recreating the in vivo-like conditions, allowing for the evaluation of the response of cancer cells to the drug. They have a strong potential in the future as further modifications can be made on the designs of the chip flexibility for better mimicking of real human tissues.

3.3. Cancer therapy

With the use of nanofluidic chips, progress in drug delivery and novel cancer treatment such as immunotherapy has been facilitated. The nanofluidic device can be used as an immunotherapeutic agent [11]. Cancer immunotherapy, unlike other forms of traditional treatment, uses the body's own immune system to identify, locate, and eliminate cancer cells. The immune system normally defends the body against infections and diseases. Nonetheless, cancer cells can occasionally avoid the immune system's recognition, allowing unchecked tumour genesis. Immunotherapy aims to overcome this evasion and enhance the immune response against cancer. Nanofluidic chips play an important role in realising and improving intratumoral immunotherapy. Unlike conventional systemic immunotherapy which undergoes subcutaneous or peritumoural delivery, intratumoral immunotherapy allows direct injection to the tumours centre, which is more effective in tumour inhibition and less detrimental to the human body.

An implantable nanofluidic chip with a size smaller than a rice grain has been invented for intratumoral immunotherapy, called NDES (nanofluidic drug-eluting seed) [11]. The NDES employs a silicon-microfabricated nanofluidic membrane to provide consistent intratumoral drug injection without external efforts such as pumps or manual control. The membrane contains slit nanochannels that regulate the diffusion of the drug from the seed to the tumour. The concentration difference of fluid inside the NDES and the tumour drives the flowing of antibodies into the tumour continuously until the antibodies in the seed are used up. Recently NDES has been tested for triple negative breast cancer and pancreatic cancer, receiving positive results [10,11]. For dealing with distinct cancers, different antibodies of different sizes are used. Different dimensions of the nanochannels are thus designed case by case to ensure the controlling over fluids flow, determined by the hydrodynamic radius of antibodies. This selection is made using a previously developed algorithm, which takes into account the channel-to-molecule size ratio. For example, in the case of treating triple negative breast cancer, the dimension of nanochannels in NDES are 20 nm, which allows for controlled and sustained release of the therapeutic antibodies CD40 whose radius is approximately 6nm [11].

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This nanoconfinement effects including electrostatic, steric, and hydrodynamic effects exerted by nanochannels are the key to regulate the fluid flow, by hindering the transport of the antibodies and resulting in a sustained release profile. By comparing anti-tumour immune response and inhibition of tumour growth between using NDES and conventional immunotherapy, NDES-facilitated intratumoural therapy has been proved to be more effective and less toxic in treating breast and pancreatic cancers, suggesting a better immunotherapeutic solution.

4. Revolutionised advantages of micro/nanofluidic in cancer study and therapy

4.1. Potential to create new cancer diagnosis method

With confinement by nanofluidic ships, analysis of single strands can be realised which cannot be done by a bulk method. The base pair information can only be reviewed when single-stranded DNAs are studied and visualised, but not double strands [9]. Base pairs information which may reveal the cancer information can thus be accessed more easily and accurately. There are other benefits in using nanofluidic screening to do tumour cells and patients' DNA analysis, such as improving transport of molecules by facilitating capillary and diffusion [5]. Techniques such as electrophoresis and electroosmosis can also be applied to the chip system, enabling precise control over the movement and separation of molecules within nanoscale channels, thus enhancing the efficiency and speed of analysis [5]. Other than saving time, nanofluidic systems also save the amount of analyte by using only minute quantities of analyte. This can not only reduce waste but also is particularly advantageous when the analyte is rare, limited and expensive.

However, there is still a big gap in using DNA analysis to do completely reliable cancer diagnosis in clinical practice. The formation of cancer cells is attributed to complicated reasons rather than merely genetic mutation. Epigenetic factors which cause tumour genesis can hardly be detected by this technique. Using single DNA to conclude cancer diagnosis is thus limited. Besides, there is a shortage of verification and evaluation of the outcome accuracy of this technique currently. Hence, it is so far suggested to be used as a reference merely.

4.2. Potential to provide a novel drug studying platform

Traditional cancer modelling and drug testing methods such as 2D cancer cultural models and xenograft models present several significant challenges. First, over simplification in biological complexity due to species differences between the host animal such as mice and the human source often confounds experimental outcomes. Moreover, the precision of measurement of tumour volume through the skin using callipers may be restricted, and thus the results may not correctly reflect the dynamic changes inside the tumour [10]. Furthermore, a considerable number of animals are required for histological examinations at various time periods. This would result in higher cost, higher experimental variability and also ethical challenges.

In contrast, cancer-on-chip technology not only has the potential to offer an alternative to traditional modelling methods but also has several advantages over them. Firstly, it accurately mimics xenograft drug responses by enabling dynamic control of solute concentration, visualising and quantifying drug concentrations in a manner akin to the changes observed in the blood of mice over time. Additional materials can be added in the chip to modify its modelling environment to approach the xenografts model. For instance, the application of chemicals like Pluronic F127 on the well surface of the chip, can prevent adhesion and non-spheroidal growth of the spheroids. This ensures the outer diameter to accurately reflect the spheroid volume, mimicking the xenografts model and enhancing the precision of measurements [10]. To simulate the conditions in real human tissues, various chemicals, techniques such as electrophoresis, more intricate design with nanochannels can be added to the chip, which has higher flexibility than using xenografts where all the conditions are restricted by the host animals.

Moreover, the cancer-on-chip does not affect the control of tumor growth and the drug effects. Rigorous analysis, including calculating the P value, has established that the chip's presence does not negatively influence cancer spheroid growth or the drug efficacy [10]. Other than providing the

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necessary response data same as other models, chips can offer additional information such as the recovered growth of cancer spheroids over time, because they are designed in the way for easier observation and more accurate measurement. This insight into growth recovery rate for cancer cells is critical in arranging future treatment such as the dosage schedules.

Overall, the cancer-on-chip model manages to include in vivo characteristics of the microenvironment of drugs and tumours, from physicochemical to biomedical aspects. Therefore, the tumour model on-chip is able to create a more representative and controlled environment to study drug efficacy compared to traditional xenograft models. It allows for the evaluation of drug response in a more physiologically relevant context and can potentially reduce the variability of in vivo assays. This method has provided more possibilities for future cancer drug study and testing.

4.3. Potential to create new treatment methods

The Nanofluidic Drug-Eluting Seed (NDES) emerges as a promising avenue for a novel cancer therapy method - intratumoral immunotherapy, surpassing conventional chemotherapy, radiation therapy and conventional immunotherapy with higher target precision to tumours, less side-effects and less toxicity, allowing patients to have a better quality of life.

Traditional methods listed above have the limitations of damaging surrounding healthy issues or causing hepatotoxicity. As an immunotherapeutic agent, NDES has solved some of the problems of intratumoural immunotherapy such as eliminating the risk of the burst amount of drug delivery. NDES leverages the concept of nanoconfinement to provide constant and sustained immunotherapeutic delivery without the need for injections or external manual intervention. The nanochannels within the NDES which only have the size of tens of nanometres, employ a variety of strategies including hydrodynamic, steric, and electrostatic effects, to control the release consistently [11]. This sustained release profile is essential for therapeutic quantities of antibodies to be present in the tumour for an extended length of time. This not only helps maximise the immune system effects but also greatly reduces the safety concern of intratumoural immunotherapy.

From the clinical operation perspective, NDES realises a sustained delivery profile, lasting for months which lowers the rate of repeated intratumoral injections by clinicians. This not only eases the patient experience but also alleviates the time and labour burden in terms of clinical operations in real-life.

4.4. Current Shortcomings and Future Challenges in Micro/Nanofluidic technology

Applications outlined in this paper have shown strong potential to micro/nanofluidic chips. However, most of the applications are still at a starting stage. Far more experimental trials and innovative explorations needed to be done to verify our predictions and expectations on them. Besides, it is essential to acknowledge that there are currently some general limitations and challenges with transforming micro-/nanofluidic technology, especially nanofluidic technology to massive use and clinical practice. These challenges underscore the need for ongoing research and innovation to harness the full potential of nanofluidic technology.

The current nanofluidic testing process on DNA or protein analysis mostly takes a relatively long time, from the fabrication process to obtaining data. There are strict and high-standard environmental conditions required for micro/nanofabrication, leading to a relatively slow manual crafting process. The small dimension of nanofluidic chips also means numerous rounds of scanning needed to be done for a 2-meter human being DNA to be tested thoroughly if needed. It is suggested that the efficiency of nanofluidic screening can be improved by combining it with other more advanced and automated techniques. For example, there was a case combining nanofluidic methods and machine learning together for pancreatic cancer cells analysis [12, 13]. More such work can be done to ease and speed up the nanofluidic analysis process.

Additionally, due to the extremely sophisticated structure of the nanofluidic device, it is challenging so far to achieve fast and large-scale production. Most of these devices can only be done in the clean room of research labs, requiring very strict fabrication conditions. The high requirements in

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nanofabrication have resulted in low reproducibility and high cost of one single nanofluidic device. There are also other problems faced by this technology such as a lack of research in relevant areas and a shortage of trials on biological bodies. There is still a long way to go to massive productions and clinical practice.

5. Conclusion

In conclusion, the advent of micro/nanofluidic chips has shown great potential in the field of cancer research and therapy. The remarkable progress made in this domain, as outlined in this paper, has not only uncovered revolutionary methods to study tumours and drugs but also innovatively utilised the unique properties of nanoscale fluidic systems to address critical challenges in cancer therapy. The application of nanofluidic chips in DNA and protein analysis has facilitated the identification of specific biomarkers, enabling early cancer prediction and personalized treatment strategies. Moreover, the use of microchips in cancer modelling and drug testing has expedited drug development processes and provided vital insights into cancer pathogenesis and drug responses, reducing the need for animal models. Last but not the least, the invention of nanofluidic drug-eluting seeds, has brought improvement in immunotherapy, minimizing its side effects and facilitating the development of new cancer treatment approaches.

However, while the potential of micro/nanofluidic chips in cancer research and therapy is undeniable, we must also acknowledge their limitations. Challenges related to scalability, mass production, and the integration of multiple functions into a single device remain. Additionally, standardization and validation of these technologies are imperative for their widespread clinical adoption.

Looking ahead, the micro/nanofluidic chip is a promising technique in cancer research and therapy. The continued evolution of these chips, together with advancements in materials science, microfabrication techniques and combinations with other techniques such as AI and automation is expected to address current limitations and further unlock their potential. With further improvement and more efforts put in this field, micro/nanofluidic chips could have become invaluable tools in the battle against cancer, bringing us more breakthroughs in cancer research and more effective and more tolerable ways to treat cancers.

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