Engineering of triboelectric-nanogenerator powered devices and lab-on-a-chip devices for biological applications

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**ENGINEERING OF TRIBOELECTRIC-
NANOGENERATOR POWERED DEVICES AND LAB-
ON-A-CHIP DEVICES FOR BIOLOGICAL
APPLICATIONS**

YANG GUANG

Ph.D.

Supervisor: Yong Ken Tye

SCHOOL OF ELECTRICAL & ELECTRONIC ENGINEERING

2019

**ENGINEERING OF TRIBOELECTRIC-
NANOGENERATOR POWERED DEVICES AND LAB-
ON-A-CHIP DEVICES FOR BIOLOGICAL
APPLICATIONS**

YANG GUANG

School of Electrical & Electronic Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Authorship Attribution Statement

This thesis contains material from 3 paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 2 is published as P. Song, **G. Yang**, T. Lang, and K.T. Yong. Nanogenerators for wearable bioelectronics and biodevices. *Journal of Physics D: Applied Physics* **52**, (2018). DOI:10.1088/1361-6463/aae44d.

The contributions of the co-authors are as follows:

- Dr. Song provided the initial project direction and wrote half of the manuscript drafts.
- I wrote the other half of the manuscript drafts.
- The manuscript was revised by Dr Lang and Prof. Yong.

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- Prof. Wang, Prof. Yong and Dr. Song suggested initial project direction and Dr. Song wrote the manuscript drafts.
- Dr. Kuang fabricated the TENG and did the calibration, and plot some figures in the manuscript
- I fabricated the implantable drug delivery devices for the experiments

- The manuscript was revised together with Dr. Panwar, Dr. Tng, Prof. Tjin, Prof. Ng, Dr.Majid, and Prof. Zhu.

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- Dr. Yang and I suggested the initial project direction and wrote the manuscript drafts.
- Dr. Yang carried out experiments of RT-PCR and western blot.
- Dr. Ouyang helped in figure generation and simulation.
- Dr. Kuang fabricated the TENG device.
- The manuscript was revised together with Prof. Wang, Prof. Yong, Prof. Zhu, Prof. Poenar, Prof. Xu and Dr. Song.

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Date

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Summary

The contact electrification is the phenomenon that when two different materials come into contact through friction, their surfaces become oppositely charged. Triboelectric-nanogenerator (TENG) was invented based on this phenomenon to effectively harness ambient mechanical energy that is omnipresent but usually not effectively utilized in daily life. Lab-on-a-chip (LOC) technology is usually referred as a device that integrates one or several laboratory functions on a single chip, with features such as low fluid volumes consumption, faster analysis and response times and better process control. In this thesis, TENG powered devices and LOC devices are engineered to realize various biomedical applications. First, a rotary TENG was utilized to harvest energy from the human body's motion to power an implantable drug delivery actuator for controlled and localized administration of drugs. Other than being used as an energy source, the output of TENG can also be utilized directly as electrical stimulations. A contact-separation mode TENG was used in a nanowires-assisted electroporation system to generate electrical stimulations which induce perforation on cancer cells, thus facilitating the transfection of siRNA without the aid of nano-carriers. This system can serve as an effective and versatile platform for high-throughput gene delivery in biological research and cancer treatment studies. In the realm of LOC devices, a biochip was developed for dual functions of preserving the viability of epithelial tissues as well as real-time monitoring the transepithelial resistance of the tissue on-chip. Last, the strengths of TENG and LOC were effectively utilized to develop a fundamentally new method for detecting trace amount of heavy metals in water. By coating the sensing surface with specific chelating agents, selective detection of lead ions was achieved.

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Nomenclature and Abbreviations

TENG	Triboelectric Nanogenerator
IDDs	Implantable drug delivery devices
LOC	Lab-on-a-chip
TEER	Trans-epithelial electrical resistance
PDMS	Polydimethylsiloxane
PMMA	poly (methyl methacrylate)
APTES	3-Aminopropyl-triethoxysilane
EDTA	ethylenediaminetetraacetic acid
PTFE	polytetrafluoroethylene
DMSO	Dimethyl sulfoxide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PBS	Phosphate buffer solution
siRNA	Small interfering ribonucleic acid
PCR	Polymerase Chain Reaction
FITC	fluorescein isothiocyanate

Chapter 1: Introduction

1.1 Motivation

With the development of information technology, the world has entered the era of “Internet of Things,” which is marked by the fact that we are surrounded by more and more electronic devices and sensors that are connected to each other. In a conservative estimation, there will be 50 billion connected devices worldwide by 2020, which is seven times of the world population [1]. People’s lifestyle has changed tremendously in the past decade as a result of the device miniaturization and cut down in costs. The future trend is toward the healthcare industry and wearable devices [2]. Success applications have been realized by wearable devices, which include monitoring heart rates/blood pressure [3], motion tracking [4], and drug diffusion [5]. However, a technical challenge has been raised as the size of devices becomes smaller and smaller; that is, how to power these devices and sensors. Batteries are the conventional energy source, but their capacity is limited, especially in the trend of device miniaturization. Smaller devices can only accommodate smaller batteries so that the duration of time for which the devices can be powered will be substantially restricted. Frequent replacement of batteries is unfavorable and probably impracticable when there are billions of devices. Especially in the case of implantable biomedical devices, the frequent need for surgeries to replace batteries would seriously reduce patients’ willingness to try these new therapies. Another disadvantage of using batteries is the problematic disposal issue because batteries contain harmful metals, such as lead and mercury [6]. They may leak out from the disposed batteries to pollute the environment. Therefore, there is a need to move onto greener and sustainable ways of powering abundant electronic devices in the era of “Internet of Things.”

The emerging triboelectric nanogenerator (TENG) technology seems to be an excellent solution to address this issue as TENG can utilize the mechanical energies in the ambient environment to generate electricity [7]. There are plenty of energies existing in our daily living environments, in the forms of human motions, vibrations, and raindrops. But these energies are usually being wasted because there are no suitable energy harvesters previously. The conventional electromagnetic generators are not effective in these low-frequency motion circumstances. However, TENG can harvest these mechanical energies effectively, even at a frequency below 5 Hz [8]. The working mechanism of TENG is a conjunction of contact electrification and electrostatic induction [9]. Contact electrification is the most common phenomenon being observed in our daily activities. Friction between materials with different dielectric properties would lead to charge accumulation on the two contacting surfaces, and these surface charges become the driving force for electricity generation in TENG. Due to the ubiquity of contact electrification phenomenon, TENG has great potential in applications. There have been successful researches that incorporate TENG into wearable devices for energy harvesting [10] or pressure sensing [11]. This Ph.D. research is motivated by the huge potential of TENG-powered devices for biomedical applications, especially for disease treatment.

Implantable drug delivery devices (IDDs) have several advantages over the conventional systemic drug administration routes, such as oral administration and intravenous injections [12, 13]. First, the drug delivery is localized so that the side effects caused by systemic drug administration is minimized. Second, IDDs can perform prolonged and controlled drug release to achieve better therapeutic effects [14]. Therefore, IDDs is a suitable alternative drug administration method against chronic diseases such as diabetes [15], ocular diseases [16] and some cancers [17]. Moreover,

the high controllability and flexibility of IDDs also allow doctors to perform precise tailoring of treatment strategies to meet the needs of different patients, leading to a move towards individualized medicine [18]. The disadvantage of IDDs is also apparent; surgery is required to implement the device. Especially in the trend of device miniaturization, smaller devices can only accommodate smaller batteries so that the device working time is limited. The frequent need for surgeries to replace batteries would seriously reduce patients' willingness to try this new drug administration strategy. The advancement in TENG technologies, such as TENG fabricated using soft materials [19], seems to be a solution to overcome this limitation because TENG might be utilized to power IDDs by harvesting sustainable biokinetic energy to realize self-powered IDD.

Gene therapy has become an emerging treatment strategy against cancer since the discovery of RNA interference (RNAi). RNAi is a post-transcriptional process to silence target gene expression by destroying its homologous messenger RNA (mRNA) using small interfering RNAs (siRNAs) [20]. siRNA is a short nucleotide sequence complementary which can bind to its target mRNA in the cytoplasm and induce subsequent degradation [21]. Due to the target gene silencing ability, siRNA has great potential in cancer therapy. Since the root cause of cancer is gene mutations, gene therapy has great potential in cancer treatment. For example, Yang et al. [22] demonstrated the inhibition of growth, migration and invasion of pancreatic cancer cells by using siRNA to down-regulate the gene expression of K-Ras, which is found mutated in 90% of pancreatic cancer patients, especially in the early stages of tumor transformations and progression [23]. The drawback restricting the popularization of gene therapy lies in the methods of gene delivery into the cytoplasm because those therapeutic molecules, such as siRNA, cannot penetrate the cell membrane [24]. The

current methods for siRNA delivery can be categorized into two strategies. One is to encapsulate siRNA into nanoparticles as a carrier to facilitate cell uptake because these carefully engineered nanoparticles can be taken by cells through endocytosis process [25]. However, the preparation of these siRNA-containing nanoparticles is time-consuming and skills-demanding because complicated synthesis and purification steps are involved. Another delivering strategy aims for direct delivery by disrupting the permeability of the cell membrane [26]. This could be achieved by applying electrical stimulations to disrupt the phospholipid patterning on the cell membrane to generate nanosized pores on the membrane (electroporation) [27]. There are also articles reporting on the use of functionalized nanowires to pierce through the cell membrane for delivering biomolecules [28]. As a high-voltage generator, TENG can be utilized to replace the bulky energy source equipment used for electroporation because the characteristics of TENG output perfectly meet the electrical needs in this application: high voltage, pulsed output and low current.

This Ph.D. research is also motivated by the development of Lab-on-a-chip (LOC) technology in exploring miniaturized, portable, robust and integrated devices for biological applications. LOC technology is usually referred to the class of technology that integrates one or several laboratory functions or operations on a single microfluidic chip, with features such as low fluid volume consumption, high-throughput analysis, fast response time and better process control. The LOC technology is first emerged on silicon wafers, fabricated using well-developed micromachining processes, to realize devices such as mixers, valves, pumps and fluidic sensors. After that, polydimethylsiloxane (PDMS) soft lithography technique was introduced into the LOC fabrication, this greatly reduced the complexity and cost of fabricating LOC devices, making them more affordable to researchers [29]. Furthermore, LOC devices

made of PDMS have excellent optical transparency and biocompatibility, making them good tools for biological studies. There are a large variety of chemical-based and optical-based LOC devices that have been demonstrated very useful for many biological applications, including cell sorting [30, 31], drug synthesis [32], single cell property study [33] and many more. However, these chemical-based and optical-based LOC devices are usually not genuinely standalone devices because they still require sophisticated equipment, such as spectrometer and epifluorescence microscope, to do the sample analysis. Electrical-based LOC devices might become “ideal” standalone devices because the electrical signals can be easily collected and sent to a computer for analysis. For example, chip-based patch clamps devices are developed to replace conventional experimental setup to study the electrophysiological properties of single cells [34]. Electrical-based LOC devices are relatively rare because their applications are usually related to single cell studies where the experimental setup is too complicated to be miniaturized and integrated on a single chip. But electrical-based LOC devices may have great potential in accommodating tissue samples as the device structure would be much simplified. Among all the applications, electrical-based LOC devices might be highly suitable for assessing the viability of tissue samples. In the conventional method to assess tissue viability, the sample is chemically stained followed by being cut into thin slices for observation under a microscope [35]. This method is time-consuming, expensive and more importantly, destructive to the tested samples. On the other hand, electrical methods may provide an alternative strategy to determine the tissue viability, which is fast in response, label-free and non-destructive.

Most heavy metals (for example, Pb, Cd, As) are deleterious even at very low concentrations because they have high tendency to form complexes with biomolecules containing nitrogen, sulfur, and oxygen, resulting in many serious consequences such

as structural changes in proteins, breaking of hydrogen bonds and inhibition of enzymes [36]. Heavy metals are not biodegradable and therefore they get accumulated in human organs, causing long term negative health effects. For this reason, maximum contaminant levels have been defined for drinking water by different organizations from all over the world. The current states of strategies for trace heavy metal detection are mainly focusing on chemical [37] and optical techniques [38]. Although these techniques demonstrate excellent sensitivity, their widespread usage is limited by some common drawbacks such as bulky and sophisticated equipment, time-consuming procedures and limitation to laboratory detection. There is a need to develop fundamentally new sensing technologies with features including fast response, simple operation and portability. By integrating the advantages of triboelectrification with LOC technology, electrical-based LOC devices might be a suitable solution towards this target.

In summary, the development of TENG and LOC technologies have opened many new applications in the biological realm. This Ph.D. research will focus on the engineering of TENG-powered devices for disease treatment and developing electrical-based LOC devices for assessing tissue viability and detecting trace heavy metals in water.

1.2 Objectives

The research works presented in this thesis include four projects:

1. The first project is to demonstrate that self-powered IDD's system can be achieved by utilizing TENG to harvest biokinetic energy. A rotary TENG is fabricated as the energy harvester to power an electrochemical microactuator to demonstrate controlled drug delivery.
2. The second project is to explore the application of TENG for electroporation. A nanowire-assisted electroporation chamber is fabricated using a facile one-step thermal treatment process for siRNA delivery. A contact-separation TENG is fabricated to provide electrical stimulation for inducing electroporation. The experiments are first carried out using fluorescein-labeled siRNA to confirm the success of gene delivery. Then therapeutic siRNA is delivered to study the cell behavior of gene silence.
3. The third project is to develop an electrical-based LOC device for assessing the viability of tissue samples. Trans-epithelial electrical resistance (TEER) is selected as the electrical parameter to be measured as it is a widely accepted quantitative technique to measure the barrier integrity of epithelial cells. It is conducted by applying an AC electrical signal across a cellular monolayer, and then measuring voltage and current to calculate the electrical resistance of the barrier. When epithelial cells become less viable, the tight junction between them is disrupted, resulting in an altered TEER value. The viability of the tissue samples is also assessed using conventional methods including MTT assay, live/dead assay and histological analysis. The aim of the project is to study whether there is a correlation between the TEER values and tissue viability.

4. The last project is to develop an electrical-based LOC device for detecting trace heavy metal in water. The working mechanism of the sensor is based on liquid-solid contact electrification between the water and the sensing area. The surface of the sensing area is functionalized with chelating molecules, which is responsible for metal recognition. The capture of heavy metals by the chelating agents alters the surface charge at the sensing area, leading to a change in electrical signals that can be detected. By choosing a specific chelating molecule, selective detection of lead ions is achieved.

1.3 Major contribution of the Thesis

The major contributions of this Ph.D. thesis include proof-of-concept demonstrations of utilizing TENG for disease treatment applications and developing two electrical-based LOC devices for tissue viability assessment and heavy metal detection, respectively. All the methods explored in this Ph.D. research have overcome certain limitations in conventional methods. The details are listed below:

1. The engineering of a fully biocompatible electrochemical microactuator for drug delivery purpose. The microactuator can be easily fabricated using currently available microfabrication techniques and controllable in pumping flow rate. The interdigitated electrodes are made of multiple layers of titanium/platinum in a sandwich pattern to address the metal delamination issue. Hence, the actuator exhibits good reliability and lifetime. (See Chapter 3)
2. The first TENG-powered drug delivery system was presented. Trans-sclera drug delivery into porcine eyes *ex vivo* was achieved with power from facile human motion. (See Chapter 3)
3. A TENG-powered nanowire-assisted electro-transfection system was developed as a versatile platform for direct gene delivery was achieved without using chemical carriers. The system was simply driven by human tapping motion and no extra energy source equipment was needed. The nanowire fabrication method is simple and low cost. As compared to the conventional electro-transfection method, TENG-powered system exhibited higher performance in terms of transfection efficiency and viability. (See Chapter 4)

4. The transepithelial resistance was proved to be correlated to tissue viability that was evaluated by multiple methods including MTT assay, live/dead assay and histological analysis. TEER was demonstrated to be an excellent method to assess tissue viability because it is easier, faster, label-free & non-destructive. (See Chapter 5)
5. A dual-function LOC device was developed for tissue culturing and on-chip monitoring of TEER. The complexity of measuring TEER of tissue is much simplified with using the LOC device. (See Chapter 5)
6. A fundamentally new method for heavy metal detection was developed based contact electrification effect. The new method is quick in response and no bulky equipment required. Selective detection of heavy metals was also demonstrated by varying the chelating agent. Therefore, this method possess huge potential to develop portable sensors that can be used to test water samples on the spot.(See Chapter 6)

1.4 Organization of the Thesis

This thesis is organized into seven chapters. Chapter 1 discusses the motivations, objectives and organization of the thesis. In the motivation section, the major driving factors behind this research are presented. In the objectives section, the research area is defined in detail and the main focus of the thesis is illustrated.

Chapter 2 reviews literature on the four operation modes of TENG and its biomedical applications. Then the state-of-the-art technologies for implantable drug delivery, electro-transfection and transepithelial resistance measurement are discussed.

Chapter 3 presents a TENG-based self-powered iDDs and demonstrate its functionality for ocular drug delivery. Pumping flow rates from 5.3 to 40 $\mu\text{L min}^{-1}$ under different rotating speeds of TENG have been realized. Indeed, the iDDs can be powered with a TENG device rotated by human hand motion. As a proof-of-concept, *ex vivo* trans-sclera drug delivery in porcine eyes was demonstrated using the TENG-based self-powered iDDs by utilizing the biokinetic energies of human hands. This work underlines the innovations in TENG-enabled self-powered iDDs for developing novel therapeutic approaches in treating chronic diseases.

In Chapter 4, a novel TENG-driven nanowire electrode arrays (T-NEA) device was developed to facilitate the electroporation-based siRNA delivery into both adhesive and non-adhesive mammalian cells. High transfection efficiency was achieved for all the six cell lines used in the experiments. After therapeutic siRNAs were electro-transfected, the bioactivity of cells was regulated in relation to the targeted gene silencing. More importantly, the T-NEA system does not produce any significant cell cytotoxicity as result of the power characters of high voltage and low current supplied by TENG.

In Chapter 5, TEER was demonstrated to be an excellent method to assess tissue viability because it is easier, faster, label-free & non-destructive, and we proved it was correlated with viability of tissue determined using MTT, histology and live/dead staining method. Besides, a dual-function biochip is developed for culturing epithelial tissues as well as measuring the tissue TEER on chip. The use of the biochip could significantly reduce the complexity of measuring TEER and make real-time TEER monitoring possible. The biochip is fabricated in lost-cost processes and more importantly, the biochip is dismountable so that it can be reused after cleaning.

In Chapter 6, an electrical-based LOC sensor was developed based on contact electrification effect. The sensor can selectively detect Pb^{2+} , with a detection limit of 0.01 mg/L (0.01ppm), which meets the guidelines for heavy metals in drinking water recommended by the World Health Organization. This fundamentally new sensing method possesses features including fast response, simple operation and portability.

Finally, chapter 7 summarizes the major contributions of this thesis, and presents the possibilities for future work and improvements in this area.

Chapter 2: Literature Review

2.1 The origin of Triboelectric Nanogenerator and its operation modes

Triboelectric nanogenerator is invented based on the conjugation effects of contact electrification and electrostatic induction. The contact electrification (CE) occurs when two different materials come into contact through friction, and their surfaces become oppositely charged. Although this is one of the most frequently experienced phenomena known for thousands of years, the fundamental principle of this effect is still being studied. Recently, Wang's group has conducted systematic experiments using Kelvin probe force microscopy to study the physics of contact electrification [39]. Their results indicate that electron transfer is the dominant mechanism for contact electrification between solid–solid pairs. Electron transfer occurs only when the interatomic distance between the two materials is shorter than the normal bonding length (typically 0.2 nm) in the region of repulsive forces. A strong electron cloud overlap (or wave function overlap) between the two atoms/molecules in the repulsive region leads to electron transition between the atoms/molecules, owing to the reduced interatomic potential barrier. The charges created on the two surfaces are termed as triboelectric charges, which become the driving force for electron flow in the attached electrodes through electrostatic induction [40]. The second term $\frac{\partial \mathbf{P}}{\partial t}$ in the Maxwell's displacement current is directly related to the output electric current of the nanogenerator [9].

$$\mathbf{J}_D = \frac{\partial \mathbf{D}}{\partial t} = \epsilon_0 \frac{\partial \mathbf{E}}{\partial t} + \frac{\partial \mathbf{P}}{\partial t} \quad (\text{Maxwell's displacement current equation})$$

Since the TENG technology was first introduced in 2012 [41], it has been continuously developing and improving. To date, large number of TENGs of various materials and different designs have been demonstrated to harvest energy from a wide range of sources, such as wind [42], human motions [43], raindrops [44] and ocean waves [45]. Based on their operation modes, these TENGs can be categorized into four groups as depicted in **Figure 2.1**: vertical contact-separation (CS) mode, lateral sliding (LS) mode, single electrode (SE) mode, and freestanding triboelectric-layer (FT) mode.

Vertical Contact-Separation Mode (CS mode): The vertical contact-separation mode is the most basic and the first developed TENG mode. As depicted in **Figure 2.1a**, two dissimilar triboelectric layers are arranged face-to-face with electrodes attached on the back sides. Due to the triboelectric effect, two triboelectric layers create oppositely-charged surfaces after the first contact. When separated, an electrical potential is created, thus freeing electrons in one electrode flow to the other electrode to build an opposite potential in order to balance the electrostatic field. Once the two layers come into contact again, the created electrical potential is neutralized and electrons flow back. Thus, the periodic contact and separation process of the two triboelectric layers induced by external mechanical motion results in an alternating current through the electrodes. This CS mode possesses advantages of simple structure, facile fabrication, robustness, and high peak output current. Moreover, the output performance can be enhanced by stacked multilayer structures. One of the disadvantages of this mode is the difficulty in properly packaging the device because the device volume varies during operation. Niu and co-workers have established a comprehensive theoretical model for this mode wherein the real-time performance of TENGs can be mathematically calculated with varied design parameters including contact surface area, triboelectric layer thickness and gap distance [40].

Lateral Sliding Mode (LS mode): This mode is similar in structure with the vertical CS mode, but the current is triggered by relative sliding between the triboelectric layers, as illustrated in **Figure 2.1b** (left). The lateral displacement of the oppositely-charged triboelectric layers along the sliding direction creates an electric field which in turn drives the electrons to flow through electrodes in order to fully balance the generated electric field. As compared to CS mode, LS mode has lower durability since the triboelectric layers get abraded due to the sliding motion. However, LS mode can give higher output power density because the charge transfer is more effective as compared to mere contact. More importantly, this mode can be modified into a disc rotation [46] or cylindrical rotation [47] TENG with a grafting structure as shown in **Figure 2.1b** (right) to yield much enhanced output. These rotation TENG devices can be very suitable for harvesting wind and water flow energy. Related theoretical studies have been carried out to understand the basic mode of the grating structured TENG [48, 49].

Single Electrode Mode (SE mode): Both CS and LS mode TENGs share a common limitation - both of the two moving triboelectric layers are required to attach to an electrode. In contrast, an SE mode TENG consists of only one triboelectric layer and one electrode. This mode significantly expands the potential applications of TENG in various circumstances where the moving objects can directly serve as the second triboelectric layer. For example, **Figure 2.1c** depicts an SE design of TENG which can harvest energy from raindrops [49]. The device simply consists of a thin polytetrafluoroethylene (PTFE) film as one triboelectric layer attached to a layer of Cu electrode. The electrode is connected to the external load and then grounded. During the falling process, raindrops become positively charged due to friction with the air. As a raindrop falls onto the PTFE layer and the contact area increases, electrons are drawn

from ground to the Cu electrode in order to balance the local electrical field. When the raindrop flows off the PTFE layer, electrons flow back to the ground. Niu et al. presented an in-depth study of this mode with systematic calculations [50]. One of the major limitations of this mode is that the power output is relatively small because of the less amount of charge transfer between triboelectric materials.

Freestanding Triboelectric-Layer Mode (FT mode): In the FT mode, the TENG usually consists of a moving object with triboelectric charges and a non-moving triboelectric layer. Both of the electrodes are attached to the non-moving triboelectric layer. **Figure 2.1d** depicts a rotary TENG in FT mode, the presence of the moving object (the rotator) creates an asymmetric electrical field distribution on the non-moving triboelectric layer, which causes electron flow between the two attached electrodes to balance the local electrical field. Thus, periodic movement of the moving object causes oscillation of the electrons between the electrodes, producing alternating current. The FT mode is considered the most effective mode with records of both the maximum power output and energy conversion efficiency [51]. Since the moving object does not necessarily have to touch the non-moving triboelectric layer during moving, this mode could be highly robust with minimal material abrasion between the contact layers.

Figure 2.1 Four operation modes of TENG and their working mechanisms. (a) Vertical contact-separation mode. (b) Lateral sliding mode [46, 47]. (c) Single electrode mode [52]. (d) Freestanding triboelectric-layer mode [53].

2.2 Materials of TENG and techniques to enhance TENG output

The output performance of TENG is highly dependent on the materials at contact and the surface structure of the contacting layers [54]. **Figure 2.2a** is a list of material in the triboelectric series. In theory, a larger gap in triboelectric property between the two materials will induce higher triboelectrification. A more detailed quantitative triboelectric series can be found in this article [55]. As described earlier, contact electrification only occurs when the two materials are very close to each other. Therefore, introducing surface nanostructures is an effective way to enhance TENG output. **Figure 2.2b** illustrates a contact-separation TENG with gold nanoparticles on the surface to enhance output. The power density is reached as high as 313 W/m^2 [56]. **Figure 2.2c** shows a flexible arc-shaped TENG which is made of polymers and metal films. Microstructures are introduced to the PDMS layer by soft lithography to enhance the performance, with a power density of 35.6 W/m^2 [57].

Figure 2.2 Materials of TENG and techniques to enhance TENG output. (a) A qualitative triboelectric series. (b) A contact-separation TENG with gold nanoparticles for output enhancement. (c) An arc-shaped TENG made of soft materials, with microstructures for output enhancement.

2.3 Power management strategies for TENG

The energy output from a TENG exhibits many characteristic features, including: 1) alternating current (AC), 2) high in voltage but low in current, 3) intermitted output with a low energy generated from each cycle of operation. These features make TENG undesirable to be directly used as an energy source in many practical applications because a direct current (DC) power supply with smooth and regular output curve is usually favorable to power most electronics. Therefore, effective power management plays an essential role in efficient energy utilization of TENG, which has always been the difficulty and bottleneck for the practicability of TENG [58].

Figure 2.3a illustrates the equivalent circuit of a conventional power management circuit to utilize TENG to light up LEDs. The TENG is first connected to a transformer to step down voltage (and step up current), followed by a bridge rectifier to convert the AC to DC current. Capacitors are connected in parallel with the LEDs to ensure a more stable voltage supply. In many other cases, the energy from TENG need to be first stored in a capacitor or rechargeable battery to build up large enough energy to power those electronics, such as pacemaker [59], sensors [60] and drug delivery system [61]. However, the use of these additional electrical components and circuits will certainly hinder the miniaturization of TENG and limit its applications. Recently, due to the advances in supercapacitor (SC) technology, researchers start to work on flexible thin-film shaped supercapacitors and integrate them with TENGs. Supercapacitor is an electrochemical energy storing device, which has very high power density and long life but very short operation time [62]. When integrated with TENGs, it can store the irregularly pulsed electricity from TENG and gives a stable and continuous current output. The advantages of TENG and supercapacitor complement each other's shortcomings. Luo et al. fabricated a type of flexible micro-supercapacitors (MSC) using a low-cost laser engraving method and the MSCs can be easily placed on top of a TENG in array to provide enhanced power management performance (**Figure 2.3b**) [63]. A single MSC has a high capacitance of approximately 10.29 mF/cm^2 when the current density is 0.01 mA/cm^2 . In another work, Pu et al. wove the flexible SC fabric and TENG fabric into a piece of wearable power textile, which can harvest and store the kinetic energy from human motion (**Figure 2.3c**) [64]. The current from

TENG is rectified by a bridge rectifier to charge the flexible SC yarn and a capacitance of 72.1 mF/cm^2 is obtained, which is adequate for powering many practical applications.

Besides capacitor, transformer is also involved in many TENG power management circuit. However, the conventional transformer is large in size and only works in high frequency. Xi et al. had developed a universal and efficient power management system for TENG without transformer, which is suitable and efficient for harvesting low frequency (1 Hz) human kinetic and environmental mechanical energy [58]. **Figure 2.3d** illustrates the equivalent circuit of the power management module. Between the switch and load resistor R , a parallel flyback diode D_1 , a serial inductor L and a parallel capacitor C are added in sequence. These electrical components form a DC-DC buck converter, which steps down voltage (while stepping up current) from its input (supply) to its output (load). The switch is realized by a voltage comparator and a MOSFET transistor (not shown in Figure 2.3d). The comparator is used to compare the rectified voltage from the TENG with the pre-set reference voltage to control the ON/OFF state of the MOSFET. When there is voltage pulse from TENG, the MOSFET is ON so that the energy is directly supplied to the inductor, capacitor and load. When the voltage from TENG is smaller than the pre-set value, the MOSFET is OFF and energy stored in the inductor and capacitor is released to maintain a relatively continuous and stable current through the load. At 1 Hz operation of the TENG, the power management module is measured to retain 80.4% of the total energy generated by TENG. In a group of comparison experiments of charging 1 mF capacitance, the voltage only increases from 4.632 V to 4.636 V after charging with a TENG directly for 5 mins, while the voltage increases from 4.632 V to 5.118 V after charging by a TENG with the power management module, exhibiting 128 folds enhancement in effective charging ability.

Figure 2.3 Power management strategies for TENG. (a) Equivalent circuit of the most conventional power managed circuit consists of transformer, bridge rectifier and capacitors. (b) Integration of thin-film supercapacitor array with flexible TENG [63]. (c) Integration of flexible SC fabric and TENG fabric into wearable power textile [64]. (d) Equivalent circuit of a buck converter power management module for TENG without transformer [58].

2.4 Application of TENG in harvesting bio-kinetic energy and biomedical applications

Efforts have been made to develop different types of nanogenerator in order to harvest bio-kinetic energy, which exist in different forms. Generally, nanogenerator for biomedical applications can fall into three types, i.e., thin film generator, wearable fiber generator and air/liquid flow energy harvester. These devices that serve as skin patch, shoe pad, wristband and implantable film are able to harvest energy from body motion or organ movement through deforming, stretching, twisting and pressing on the film (**Figure 2.4 a-g**) [43, 65-72]. Implantable device is a rapidly rising medical technology in recent years, yet it has always been difficult to power implantable devices. Implantable thin film nanogenerator offers a viable solution to harvest mechanical energy from heart beating or eye balls rotating and to power microdevices nearby (**Figure 2.4d and 2.4g**) [66, 67]. Fiber-based nanogenerators has aroused many attentions recently for their applications in smart fabrics such as clothes, socks and hats, making energy harvesting easier and user friendly in people's daily life [73]. Besides, human circulation system contains non-stop flowing fluid that preserves sustained bio-kinetic energy (**Figure 2.4e and 2.4i**) [74]. Nanogenerators to harvest energy from bloodstream and breath were demonstrated. By investigating the flutter mechanics on hummingbird's wing, Ahmed et al. developed a hummingbird-inspired TENG (**Figure 2.4h**), and they oscillate upon encountering air flows to generate electrical current. Thus the device is able to harvest bio-kinetic energy from human breath for self-powered applications [75]. In the study of Xu et al., a fluidic nanogenerator fabricated from aligned multi-walled carbon nanotube (MWCNT) fibers was developed (**Figure 2.4i**) [76]. When human blood flows in the device channel, a potential is built between the input and output due to electrical double layer effect that forms a diffusion layer.

Figure 2.4 Methods to harvest bio-kinetic energy: (a) Nanogenerators as skin patch [72]. (b) wearable fabric [70] (c) TENG as a shoe pad. [69] (d) A biodegradable TENG works by implantation on heart. [42] (e) TENG in harvesting respiration energy. [74]. (f) Energy harvesting on skin.[71] (g) Self-powered heart rate sensing.[67] (h) A self-adaptive TENG to harvest wind energy. [75] (i) energy harvesting in flowing liquid [76]

TENG have been extensively explored in the realm of biomedical applications of including healthcare monitoring [67, 77], bio-sensing[77], drug delivery[78] and tissue/organ's electrical stimulation[79]. Direct supplying electrical power onto live cells, tissues and organs can effectively treat chronic pain, accelerate wound healing, control cell differentiation and stimulate neuron cells and muscles, etc. These applications do not require high electrical power. For instance, only a few micro joules (mJ) of electrical power would be sufficient for triggering the action potential of cardiac myocytes, but continuous treatments are usually highly required. Wearable electronics or implantable systems are considered promising for conducting electrical treatments anytime and anywhere outside the clinic or home, making the process more convenient and acceptable to patients. Indeed, the synergistic developments of wearable electronics and nanogenerators technologies make home-based electrical therapy more likely to come true. By direct converting ambient mechanical energy into electrical energy onto targets, nanogenerator technologies have linked energy harvesting with treatment process seamlessly. Hwang et al. first demonstrated the usage of flexible single crystalline TENG as a self-powered artificial cardiac pacemaker (**Figure 2.5a**) [79]. The TENG harvested bio-kinetic energy from biomechanical motions and offered a high output current of 0.223 mA and voltage of 8.2 V to stimulate cardiac myocytes. Aiming at the same application, Zheng et al. reported an implantable TENG (iTENG) to harvest energy from breath driven motions of animal thorax (**Figure 2.5b**) [66]. Their work on biodegradable TENG (BD-TENG) have further enhanced the bio-compatibility of implanted TENG to ensure the safety of the treatment (**Figure 2.5c**) [80]. In other approaches, researchers exploited the harvested energy to stimulate live cells. In particular, the stimulation of live neuron cells is of great significance for developing artificial nerve system and recovering damaged neuron tissues. Hwang et

al. reported an implantable nanogenerator for real-time deep brain stimulation in a mice's motor cortex to induce forearm movements on the mice (**Figure 2.5d**) [81]. Guo et al. reported the study of TENG together with extracellular microfibers matrix to promote the differentiation of mesenchymal stem cells into neurons (**Figure 2.5e**) [82]. TENG's practical use as a wearable stimulation system to assist nerve regeneration is demonstrated on a walking person [82].

Figure 2.5 Self-powered electrical treatment on cells, tissues and organs. (a) A flexible single crystalline TENG as a self-powered artificial cardiac pacemaker.[79]. (b) The implantation of iTENG.[66]. (c) BD-TENG degrades during the 18 days test.[80] (d) real-time deep brain stimulation in a mice's motor cortex using a TENG.[81] (e) TENG together with extracellular microfibers matrix to enhancing neural differentiation of mesenchymal stem cells for nerve regeneration.[82]

2.5 Implantable Drug Delivery Micropumps

In order to address the previously mentioned challenges for 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. Drug delivery to particular tissues is achieved through a long cannula attached to the outlet of the device's reservoirs. Therefore, drug flows can be precisely guided to diseased areas. 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 [83]. In comparison to 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 the device and the implantation processes. To date, these devices have been studied for *in vitro* [84], *ex vivo* [85] and *in vivo* [86] 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 displacement for pumping, and (ii) Non-mechanical pumps like electro-osmotic, electrowetting, and capillary pumps that rely on surface tension forces [85-86]. 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 [87]. 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 which generate gases that provide driven forces on drug solutions (e.g. via a membrane). 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 [88]. Gases were enclosed inside the bellow while at the same time pneumatic forces deflect the bellows to drive drug solutions [89]. 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.

2.6 Gene delivery with the aid of nanowires

RNA interference (RNAi) regulatory pathway was discovered in 1998, and it has opened unprecedented opportunities to investigate the bio-function of specific genes and to explore new treatment strategies against many diseases, such as hereditary diseases, viral infection, neural disorders, and cancers [90]. RNAi is a post-transcriptional process to silence target gene expression by destroying the homologous messenger RNA (mRNA) [91]. This process can be triggered by small interfering RNAs (siRNAs), which is a short nucleotide sequence complementary to its targeting mRNA. In the cytoplasm, siRNA will incorporate into a nuclease complex, followed by identifying the target mRNA and binding to it. Subsequently, the bound mRNA is cut by the nuclease complex and degrade [21]. Due to the target gene silencing ability, siRNA has great potential in cancer therapy. For example, Yang et al. [22] demonstrated the inhibition of growth, migration and invasion of pancreatic cancer cells by using siRNA to down regulate the gene expression of K-Ras, which is found mutated in 90% of pancreatic cancer patients, especially in the early stages of tumor transformations and progression [23]. In another example, Zhi et al. [92] used siRNA to inhibit the expression of matrix metalloproteinase-2 (MMP-2) in pancreatic cancer cells and subsequently observed significant decrease in the invasiveness and adhesion of the cancer cells.

Theoretically, when using appropriately designed siRNA, the RNA interference machinery can be exploited to silence nearly any genes in the body [93]. This makes siRNA promising in cancer treatment. However, the characteristics of siRNA, such as its negative charge, macromolecular structure and easy-degradable nature, impede its permeability across the cell membrane to perform its function in the cytoplasm. Hence, delivering siRNA across the cell membrane with high efficiency is a challenge in

biological and medical research. A large variety of delivery systems have been developed to overcome the barriers in siRNA transportation. Chemical transfection reagents (lipofectamine [94, 95], cationic polymers [96, 97], inorganic nanoparticles [98]), biological reagents (cell penetrating peptides[99], and viral vectors[100]) have been widely explored to improve the cellular uptake efficiency to siRNA. Briefly, nanoparticles are synthesized and encapsulate the vulnerable siRNA drugs inside to protect them from being degraded. The surface of the nanoparticle is hydrophobic to facilitate the cell uptake and the surface also can be functionalized with variety of ligands for targeted delivery and additional functions [101]. For example, Tng et al. [102] developed a multifunctional polymeric nanoparticle for anti-cancer drug delivery. The nanoparticles encapsulate paclitaxel, a chemotherapy drug, and rhodamine 6G, a fluorescent dye, inside and their surfaces are coated with gadolinium chelates, a magnetic resonance imaging (MRI) contrast agent. Hence, the delivery of drugs using this nanoparticle carrier can be imaged by fluorescence microscopy *in vitro* as well as by MRI *in vivo*. In a report of siRNA delivery, Lin et al. [103] adopted cationic polylactides rich in amine groups to form nanoplexes with Interleukin-8 (IL-8) siRNA for the protection and delivery of IL-8 siRNA into pancreatic cancer cells. The transfection efficiency of *in vitro* test was high and the proliferation of cancer cells was significantly inhibited.

Although the chemical methods can achieve some good results, yet they often have drawbacks such as time-consuming and skills-demanding in nanoparticle synthesis and immune-toxicity issues. Rather than biochemical methods that are often accompanied by endosomal degradation problems, more direct approaches, which is to increase membrane permeability by disrupting the cell membrane physically, have been developed. The most common one is electro-transfection based on electroporation

[104-109]. Compared to biochemical delivery strategies, electroporation approach progressed rapidly in recent years, benefitting from their capability to achieve direct and localized drug delivery to the desired intracellular locations [104]. Electrotransfection is often favorable for gene delivery because of its handiness, high transfection efficiency, operational convenience, and universal applicability on broad cell types [107]. In the electro-transfection process, short, high-voltage electrical pulses are applied to overwhelm the cell membrane capacitance. A perforation event happens as a result of the disruption of the cell membrane, making the perforated cells transiently permeable so that the charged biomolecules (such as drugs, proteins or genes) can enter the intracellular locations by the electrophoretic movement [110]. The formation of pores requires threshold electrical energy, and the biomolecular movement driven by the electrophoretic effect depends on both the pulse strength and duration. Currently, the conventional bulk electroporation technique is performed at high voltage (0.5~1.0 kV/cm pulse strength in many protocols) with cells in suspension status and consumes a large number of biomolecules [106]. Although the permeability of the cell membrane could be effectively enhanced, the involvement of high voltage pulses may inevitably cause irreversible cell damage and even cell apoptosis. Thus, the high cell viability and high transfection efficiency are hard to be achieved simultaneously, a compromise between acceptable transfection efficiency and cell viability is always dilemmatic. Hence, the structure of the electroporation device and the supplied power are required to be optimized for overcoming these issues.

Recently, novel electroporation devices with micro and nanoscale structures have been developed to overcome these challenges, including closely patterned nano-electrode pairs[108, 109, 111]and micro/nanofluidic channel constriction [112, 113]. Lee and co-workers have fabricated electroporation devices where individual cells are

sandwiched between two nanofibers supported porous sheets to achieve uniform electric field across the cells. [114, 115]. Unlike conventional bulk electroporation, this system directly drives DNA molecules into the in-situ growing stem cells, thereby improving gene transfection efficiency. However, non-uniform cell-to-substrate contacts diminish the transfection yield at acceptable cellular viability [116]. Recently, devices with vertical nanowire arrays have been developed as a universal platform for intracellular siRNA delivery into a broad range of cell types through direct cell membrane penetration [108, 109, 117]. Yet, in these cases, the stochastic nature of cell penetration makes it difficult to achieve temporal and dosage control over the delivered biomolecules, and cell elution from the nanowires is also problematic. Melosh group introduced a hollow nanostraw (about 100nm in diameter) system that can achieve drug intracellular delivery with precisely temporal and spatial control. The small nanostraw can spontaneous penetrate the cell membrane to realize direct intracellular delivery[108]. However, the overall transfection efficiency is still less than 10%. Moreover, the connections between nanostraws and cytoplasm produce leakage channel for ions and proteins, and this may alter the biological behavior of the cells. Cui group developed one-dimensional nanomaterial-assisted electroporation using static electricity as a power source to efficiently inactivate both bacteria and viruses through membrane damage. The advantages of this disinfection method include low energy consumption and no harmful byproducts generated [118]. The 1D nanopillar structure enhances the electric field at the vicinity of nanopillar tip based on the “lightening rod effect” so that the applied voltage for electroporation was reduced to be only several volts [119]. The “lightening rod effect” happens when a thin metallic wire is placed in a uniform electric field, the electric field strength at the vicinity of metallic wire tip can be greatly amplified by a multiplier which is approximately proportional

to the ratio of the wire length over diameter. Wang group developed a new micropillar array electroporation (MAE) platform to advance the electroporation-based delivery of DNA and RNA into mammalian cells [120]. By fabricating well-patterned micropillar structures on the electrode surfaces, the electric field strength near the micropillar was significantly enhanced, leading in three times efficiency increase on plasmid DNA transfection, and a 10–55% increase in gene knockdown efficiency after siRNA transfection.

Another physical delivery method is to utilize nanoscale material with high aspect ratio to poke into the cytoplasm for direct intracellular delivery. For example, Yoo et al. [109] utilized a gold nanowire (100-150nm in diameter) to poke into cells for DNA delivery. The manipulation of nanowire is done by using the tip of atomic force microscope (AFM) and the binding and release of DNA molecule is controlled by applying positive and negative charge on the tip respectively. Because the diameter of the nanowire is 2 orders of magnitude smaller than the size of cell (around 10 μ m), the viability of the poked cell is not much affected. Nanoscale materials with high aspect ratio have the potential to become an ideal strategy for delivering gene molecules into live cells with the following characteristics: 1) minimal perturbation to cell functions; 2) capability to delivering variety of biomolecules into a wide range of cell types; 3) high throughput transfection performance [110]. Vertically aligned silicon nanowire substrate (SiNWs) is a good candidate to meet these requirements. Si is low-toxic and compatible with cell culture.

A few researchers reported that various types of cells such as immortal cells, human primary cells and rat neuron cells could be cultured on vertical SiNWs and cell proliferation of immortal cells was observed [111]. Action potential generation in neuron cells was also observed by Robinson et al [112]. More importantly, the vertical

SiNWs can spontaneously penetrate the cell membrane to achieve physical access into the interior of cells without external force [113]. Additionally, the relatively reactive chemical property of silicon ensures that it can be easily functionalized to be loaded with diverse biomolecules. These properties enable SiNWs to be a good candidate as a universal platform for delivering a variety of biomolecules into various tumor cells as well as primary cells. For example, Park's group [114] demonstrated using vertical SiNWs to deliver siRNA, DNA, protein and peptide into HeLa cells, human fibroblasts and rat progenitor cells. In another research work, Park's group [115] utilized SiNWs platform to knockdown genes to investigate the potential basis of clinical heterogeneity in chronic lymphocytic leukemia by knocking down genes involved in the Wnt signaling pathway (**Figure 2.6**). Besides these in-vitro applications, in-vivo experiments have also been carried out. For example, Chiappini et al. [116] utilized porous SiNWs to transfect VEGF-165 gene in mice and managed to induce neovascularization at the region of target. It is also notable that the porous SiNWs used is biodegradable.

Figure 2.6 SiNWs successfully deliver LEF1 siRNA into ex vivo human B cells obtained from Chronic Lymphocytic Leukemia (CLL) patients. (a) SEM image of CLL-B cells on top of NWs taken 24 h after plating. (b) Confocal images of CLL-B cells on SiNWs loaded with three different siRNAs (non-targeting control siRNA, LEF1 siRNA and cell death-inducing siRNA) taken 24 h after plating. Administering a cell death-inducing siRNA kills a larger number of cells than a non-targeting control (cytoplasm in live cell: green, dead nuclei: magenta). The middle sample shows the effect of LEF1 siRNA on CLL-B cell viability for one particular patient sample.

Chapter 3: Implantable Drug-Delivery System Powered by a Rotary TENG Harvesting Biokinetic Energy

3.1 Introduction

Implantable drug delivery devices (IDDs) have several advantages over the conventional systemic drug administration routes, such as oral administration and intravenous injections [12, 13]. First, the drug delivery is localized so that the side effects caused by systemic drug administration is minimized. Second, IDD can perform prolonged and controlled drug release to achieve better therapeutic effects. Therefore, IDD is a suitable alternative drug administration method against chronic diseases such as diabetes, ocular diseases and some cancers. Moreover, the high controllability and flexibility of IDD also allow doctors to perform precise tailoring of treatment strategies to meet the needs of different patients, leading to individualized medicine [18]. The disadvantage of IDD is also apparent; surgery is required to implement the device. Especially in the trend of device miniaturization, smaller devices can only accommodate smaller batteries so that the device working time is limited. The frequent need for surgeries to replace batteries would seriously reduce patients' willingness to try this new drug administration strategy. Recently, there are TENGs being fabricated using soft materials [19]. These TENGs might be utilized to power IDD by harvesting sustainable biokinetic energy.

In this chapter, the proof-of-concept TENG-powered IDD for drug-delivery applications is presented. The entire system consists of a rotary TENG and an implantable drug delivery microactuator. As sketched in **Figure 3.1**, the TENG output is first transformed and rectified to direct current, and then connected to electrodes of the drug delivery microactuator. When voltage is applied, the electrochemical

electrodes would induce hydrolysis reaction to generate gases which in turn drive the drug out of the microtube. The entire IDD is covered in polydimethylsiloxane (PDMS) to grant biocompatibility. The performance of the IDD was characterized with TENG powering. As a demonstration, trans-sclera drug delivery was performed in porcine eyes *ex vivo*.

Figure 3.1 Structural designs of TENG and the IDD. (a) Schematic illustration of the TENG-based iDDS. (b) Schematic illustration of the detailed design of TENG. (c) Schematic illustration of the detailed design of IDD.

3.2 Materials and Methods

3.2.1 Fabrication of TENG:

The TENG was composed of three layers (**Figure 3.1a**): i) a layer of metal Cu patterned with radial-arrayed strips as the rotator, ii) a layer of PTFE film, and iii) another layer of metal Cu patterned with two sets of complementary radial-arrayed electrodes as the stator. The metal structure was fabricated by using a standard printed circuit board technology that was reported previously.[18] Two wires were connected to the two complementary electrodes on the stator respectively. The PTFE film was flattened and pasted on the layer of Cu electrodes.

3.2.2 Fabrication of the IDDs

The IDD consisted of three parts (**Figure 3.1c**): i) an electrochemical pressure pump, ii) a PDMS drug reservoir, and iii) a long PDMS microtube. The pressure pump was fabricated by using standard photolithography and e-beam metal-deposition method to achieve the interdigital Au/Ti electrodes on a silicon wafer, as mentioned in our previous publications.[7,26,37] The drug reservoir was fabricated by using PDMS soft lithography method on a 3D printed-ABS mold. The dimension is 10 mm long, 10 mm wide, and 2 mm high.

3.2.3 Fabrication of the PDMS microtube

The long tube was made by heat curing of PDMS coated on a Tungsten wire. Dip a piece of Tungsten wire into uncured PDMS mixture and then pull the wire to make it straight. A small current was applied through Tungsten wire to heat it until a very thin layer of PDMS on the wire surface is cured. Then the cured PDMS and wire are put in ethanol for 30 mins sonication before the wire can be put out from the as-

formed PDMS tube. The tube has the same inner diameter as the Tungsten wire diameter, which is 0.3 mm.

3.2.4 Measurement

Measurement: The TENG was driven by a motor. The output voltage and current of the TENG were measured by low-noise voltage preamplifiers (Keithley 6514 System Electrometer and Stanford Research SR570). The drug-delivery flow rates under TENG rotation at different speeds were measured. The volume of water delivered from the device in 60 s was calculated by measuring the liquid moving distance in a PDMS microchannel.

3.2.5 Implantable Drug Delivery Micropumps

Ex Vivo Ocular Drug-Delivery Study: Enucleated porcine eyes were used to conduct the initial ex vivo studies. The study protocol was approved by the institutional animal care and use committee (IACUC) at Nanyang Technological University (ARF-SBS/NIE-A0312). A minimally invasive surgical technique was developed to insert the PDMS microtube in the anterior chamber of the eye to achieve trans-sclera drug delivery; drug treatment in this location is needed for treating ocular diseases such as glaucoma. In detail, a 1 mm incision was made on the sclera, then an 18 gauge needle was used to create a tunnel to anterior chamber. A PDMS microtube was inserted into the anterior chamber through the tunnel. Following microtube implantation, the self-powered functionality of the device was tested by operating the human-hand-driven TENG. Fluorescent polystyrene microparticles ($\approx 2 \times 10^7$ particles/mL, Fluoresbrite YG Microspheres $\approx 5.7 \mu\text{m}$, Polysciences Inc.) in DI water were used to trace the drug perfusion in eye. After delivery, the porcine eyes were imaged under an inverted microscope.

3.3 Design and working principle of the TENG

Figure 3.2a is the image of the as-fabricated rotary TENG. The rotary TENG is selected in the project because it can be easily operated by both human and a motor, so that the performance of the IDD can be characterized with consistent input when the TENG is powered by the motor. The TENG was composed of three layers (**Figure 3.1b**): i) a layer of metal Cu patterned with radial-arrayed strips as the rotator, ii) a layer of polytetrafluoroethylene (PTFE) film, and iii) another layer of metal Cu patterned with two sets of complementary radial-arrayed electrodes as the stator. **Figure 3.2b** sketches the detailed electrodes pattern on the stator. The PTFE film was pasted in between the two metal layers as the electrification material. **Figure 3.2c** illustrates the working principle of this freestanding mode TENG. Briefly, upon rotation, the Cu grating on the rotator becomes positively charged due to the contact electrification with the PTFE film, while negative charges build up on the PTFE film. On the PTFE film, at the locations where the Cu grating is present, the positive and negative electric fields neutralize each other to reach an overall neutral electric field. While at the locations where the Cu grating is not present, there is a local negative electric field on the PTFE. This asymmetric electrical field distribution on the PTFE film causes electrons flow between the two complementary radial-arrayed electrodes on the stator to balance the local electrical field. Then when the Cu grating rotates forward, the electrons flow back. Thus, continuous rotation of the rotator would cause oscillation of the electrons between the electrodes on the stator, producing alternating current.

Figure 3.2 Working mechanism of the rotary TENG. (a) The image of the as-fabricated rotary TENG. (b) Illustration sketch of the two complementary radial-arrayed electrodes on the stator. (c) Illustration of the electron flow when the rotator is moving.

3.4 Design and working principle of the IDDs

The as-fabricated IDD is shown in **Figure 3.3a**, consisting of three parts: i) an electrochemical pressure microactuator, ii) a PDMS drug reservoir, and iii) a long PDMS microtube. The pressure microactuator has a design as illustrated in **Figure 3.3b and 3.3c**. The patterning was achieved by using standard photolithography and the metal deposition was conducted in an E-beam evaporator. The interdigitated electrodes are made of multiple layers of titanium/platinum in a sandwich pattern to address the metal delamination issue. Hence, the actuator exhibits good reliability and lifetime. The microactuator was sealed in the drug reservoir which is biocompatible. The working mechanism is illustrated in **Figure 3.3d**, which is to use electrical power to induce water splitting. During experiment, the output of the TENG was transformed and rectified to DC current before it was applied onto the Au electrodes for the electrochemical pressure pumping. This ensured a continuous flow of electrons to the cathode, making it negatively charged and the anode electrode positively charged. The potential built between the Au electrodes initiated the water splitting process, generating oxygen at the cathode and hydrogen at the anode. These generated gases will pump the drug out of the microtube. Reactions take place at the anode and cathode are the following:

Figure 3.3 Design and working principle of the IDD. (a) photo of the as-fabricated IDD. (b) Interdigital design of the electrodes. (c) Multiple layers of titanium/gold in a sandwich pattern to address the metal delamination issue. (d) Schematic illustration of the working principle of the electrochemical pressure pumping and the amplified photograph of the gold electrodes air bubbles generated from.

3.5 Characterization of TENG

The open-circuit voltage and short-circuit current of TENG were measured and shown in **Figure 3.4a and 3.4b**. During the experiment, the output of the TENG was transformed and rectified to DC current before it can be utilized. **Figure 3.4c and 3.4d** show the voltage and current when the TENG is rotated by hand.

Figure 3.4 Characterization of TENG output. Open-circuit voltage (a) and short-circuit current (b) of TENG under the rotating speed of 500 rpm. Transformed and rectified voltage (c) and current (d) of TENG under the hand rotating mode

3.6 Characterization of IDD

Figure 3.5a and 3.5b are the photos of the IDD before and during drug pumping. The controlled drug-delivery flow rate was demonstrated by varying the rotation speed of the TENG, as shown in **Figure 3.5c**. When the rotating speed was 300 rpm, the output voltage of the TENG reached about 5 V and the delivery flow rate was about 5.3 $\mu\text{l}/\text{min}$. Flow rate of 40 $\mu\text{l}/\text{min}$ at 600 rpm.

Figure 3.5 Pumping performance of the IDD when powered by TENG. (a) Photograph of the drug reservoir before pumping. (b) Photograph of the pressurized drug reservoir during pumping. (c) Pumping flow rate as a function of the rotating speed of TENG. Data are presented as mean $\pm$ SD (N = 5)

3.7 Ex Vivo Trans-Sclera Drug Delivery

As a proof of concept, the self-powered IDD was tested with enucleated porcine eyes *ex vivo*. The PDMS microtube was used to penetrate the physiological barrier in ocular drug delivery. A 1mm incision was made on the sclera and the PDMS microtube of the device was inserted into the anterior chamber through the incision (**Figure 3.6a and 3.6b**). This implantation method has been widely used in commercial glaucoma valves (e.g. Ahmed Glaucoma Valve, New World Medical Inc.) and also employed in other ocular drug-delivery studies [9,29]. After implantation, the drug pumping was started by hand operation on TENG to deliver 50 μ L of micrometer-sized fluorescent particles suspension into the anterior chamber of porcine eyes. The microparticles offer a suitable model to simulate the controlled release of drug carriers and enable fluorescent imaging to trace the delivery [30]. The eye's anterior chamber was then imaged to confirm the success of microparticles delivery (**Figure 3.6d and 3.6e**). Compared to topical eye drops of large volumes, the trans-sclera drug delivery requires smaller volumes of drug and generates sufficient effects for treating eye diseases such as glaucoma [31]. Clinicians suggest 50–500nL of daily dosage for sustained treatment, which renders the TENG-based self-powered IDD with prolonged usability for at least 500 days [32]. This daily dosage is much smaller than the injection volume that could cause rise in intraocular pressure [33]. Moreover, the refillability of the drug reservoir further enhances the working life of the proposed device.

Several IDD have been investigated for highly efficient ocular drug delivery [9,29]. However, powering such devices is extremely difficult due to their limited sizes and the clinical restrictions on post-device implantation. In our approach, we offer a TENG-based solution to harvest the endless biokinetic energy for powering the implantable device. This method totally bypasses the need for external power sources

(e.g. battery). Our study shows that the self-powered IDD could be a viable option to overcome the difficulties in both efficient drug treatment and sustained operation. Having concluded this, we would like to emphasize the developments of TENG that are contributing to advances in designing implantable treatment devices. First, the further miniaturization of TENG has enabled the feasibility of a fully implantable treatment device. The implantable TENG and wearable TENG is able to harvest energy directly from organs/tissues' vibrations or slight body motions [2,22]. Second, recent reported technology in soft and deformable microneedles device can be combined with the concept of self-powered drug delivery to enable ease-of-use, surgery-free, and battery-less treatment devices [34]. Third, multifunctional micromedical devices have drawn researchers' attention [35]. The noteworthy potential of TENG-based devices in biosensing (e.g. pressure sensing and droplet sensing) has also been investigated [36]. We foresee this unique multifunctional feature of TENG as a major benefit for designing a much smarter IDD. TENG could potentially be used in monitoring eye pressures in glaucoma treatment while providing a continuous power for ocular drug delivery for regulating high eye pressure.

Figure 3.6 Ex vivo drug delivery study of the TENG-based self-powered IDD. (a) Schematic illustration of the device implantation for drug delivery in the anterior chamber. (b) Photograph of the experiment after implantation surgery. (c) Amplified photograph of the anterior chamber after PDMS microtube implantation. The white arrow indicates the microtube insertion point. (d, e) Fluorescent microscopy of the anterior chamber before and after the delivery of fluorescent microparticles. Scale bars represent 200µm.

3.8 Conclusion

In summary, the recent concept of TENG has been utilized for developing a small size, batteryless implantable treatment device. We present the first TENG-based self-powered IDD and demonstrate its functionality for ocular drug delivery. Pumping flow rates from 5.3 to 40µL min⁻¹ under different rotating speeds of TENG have been realized. Indeed, the iDDs can be powered with a TENG device rotated by human hand motion. As a proof-of-concept, we demonstrate the *ex vivo* trans-sclera drug delivery in porcine eyes using the TENG-based self-powered IDD by utilizing the biokinetic energies of human hands. This work underlines the innovations in TENG-enabled self-powered IDD for developing novel therapeutic approaches in treating chronic diseases.

Chapter 4: Nanowire-Assisted Gene Electro-Transfection System Driven by Human-motion Operated TENG

4.1 Introduction

Gene therapy has become an emerging treatment strategy against cancer since the discovery of RNA interference (RNAi). RNAi is a post-transcriptional process to silence target gene expression by destroying its homologous messenger RNA (mRNA) using small interfering RNAs (siRNAs) [20]. siRNA is a short nucleotide sequence complementary which can bind to its target mRNA in the cytoplasm and induce subsequent degradation [21]. Due to the target gene silencing ability, siRNA has great potential in cancer therapy. Since the root cause of cancer is gene mutations, gene therapy has great potential in cancer treatment. For example, Yang et al. [22] demonstrated the inhibition of growth, migration and invasion of pancreatic cancer cells by using siRNA to down-regulate the gene expression of K-Ras, which is found mutated in 90% of pancreatic cancer patients, especially in the early stages of tumor transformations and progression [23]. The drawback restricting the popularization of gene therapy lies in the methods of gene delivery into the cytoplasm because those therapeutic molecules, such as siRNA, cannot penetrate the cell membrane [24]. The current methods for siRNA delivery can be categorized into two strategies. One is to encapsulate siRNA into nanoparticles as a carrier to facilitate cell uptake because these carefully engineered nanoparticles can be taken by cells through endocytosis process [25]. However, the preparation of these siRNA-containing nanoparticles is time-consuming and skills-demanding because complicated synthesis and purification steps are involved. Another delivering strategy aims for direct delivery by disrupting the permeability of the cell membrane [26]. This could be achieved by applying electrical

stimulations to disrupt the phospholipid patterning on the cell membrane to generate nanosized pores on the membrane (electroporation) [27]. There are also articles reporting on the use of functionalized nanowires to pierce through the cell membrane for delivering biomolecules [28]. As a high-voltage generator, TENG can be utilized to replace the bulky energy source equipment used for electroporation because the characteristics of TENG output perfectly meet the electrical needs in this application: high voltage, pulsed output and low current.

Recently, direct cell membrane penetration by small-diameter nanowires also has been developed as universal platforms for intracellular siRNA delivery into a broad range of cell types [108, 109, 117]. Yet, even in these cases, the stochastic nature of cell penetration and molecular elution from the nanowires complicate control over the temporal and dose of delivered biomolecules. The Melosh Group reported a nanostraw-electroporation (hollow nanowire) system that provides excellent time-resolved and spatially controlled intracellular delivery relying on the spontaneous penetration of nanostraws into cytoplasm [108]. However, the DNA plasmid transfection efficiency is generally less than 10%. Moreover, the connection between nanostraw and cytoplasm remains open after the delivery event. The biological behavior could be influenced by protein and ion leakage through these channels. Cui group developed one dimensional nanomaterial-assisted electroporation using static electricity as a power source to efficiently inactivate both bacteria and viruses through membrane damage. This system consumed low energy, and without generating harmful disinfection byproducts [118]. The 1D nanopillar structure enhance the electric field at the vicinity of the nanopillar tip based on the “lightening rod effect”, which reduced the applied voltage to only several volts [119].

Chapter 4: Nanowire-Assisted Gene Electro-Transfection System Driven by TENG

The Wang Group developed a new micropillar array electroporation (MAE) platform to advance the electroporation-based delivery of DNA and RNA into mammalian cells [120]. By introducing well-patterned micropillar array textures on the electrode surface, the transmembrane potential near the micropillar is much higher than without the micropillar. The micropillar structure contributed to a ~3-fold increase on plasmid DNA transfection and an additional 10–55% transgene knockdown with siRNA. Hence, Cui's and Wang's studies provide us with an inspiration the nanowires structure which can be used to reduce the supply power on gene electro-transfection. For this purpose, we fabricated the nanowire electrode array (NEA) for amplifying the applied electric field that was expected to benefit the siRNA transfection and reduce the cell damage.

Herein, we fabricated a T-NEA (TENG-driving Nanowires Electrode Array) platform for efficient intracellular siRNA on various cell lines. Because the electric field can be amplified by the nanowire electrode owing to geometrical sharp tips, short duration electrical pulses with low current supplied by TENG can induce transient membrane permeability and drive siRNAs into the cytoplasm. The high siRNA transfection efficiency and low damage to cell viability were achieved on adherent and non-adherent cells. After therapeutic siRNA were delivered into the pancreatic cancer line (MiaPaCa-2) and leukemia cell line (K562), the expression of targeted mutation genes (K-ras and *bcr-abl*) were effectively down-regulated on MiaPaCa-2 cells and K562 cells, respectively. The changes of bioactivity of treated cells were systematically evaluated because of the silencing of targeting mutation genes. Overall, our findings suggest that the versatility, efficiency, and uniformity of the T-NEA system can serve as a powerful and reliable platform for high-throughput siRNA intracellular delivery.

4.2 Materials and Methods

4.2.1 Cells and siRNA sequences

Three customized siRNAs (silencing K-ras, bcr-abl and non-targeting respectively) were purchased from Shanghai GenePharma Co., Ltd (Shanghai, P.R. China). The sequences of the three siRNAs are: bcr-abl: 5'-GCAGAGUUCAAAAGCCCTT-3' (sense), 5'-GGGCUUUUGAACUCUGCTT-3' (antisense); K-ras: 5'-GUUGGAGCUUGUGGCGUAGUU-3' (sense); 5'-CUACGCCACAAGCUCCACUU-3' (antisense strand); negative control siRNA (NC-siRNA): 5'-UUCUCCGAACGUGUCACGUTT-3' (sense), 5'-ACGUGACACGUUCGGAGAATT-3' (antisense). The pancreatic cancer cell line (MiaPaCa-2), leukemia cell line(K562), cervical cancer cell line (Hela cell), human hepatocellular carcinoma cell line (HepG-2), human lung cancer cell line (A549) and mouse breast cancer cell line(4T1) were obtained from American Type Culture Collection (ATCC).

4.2.2 Fabrication of vertical Nanowire Electrodes Array (NEA)

Copper mesh (10 × 10 cm) was first washed with ethanol to remove any adhesive dusts, followed by being oxidized in muffle furnace (Nabertherm GmbH, Germany) at 500 °C for 4 hours. The fabricated nanowires on the surface of the copper mesh were observed with a SEM (Thermo Scientific™ Scios™ DualBeam™ System). The processed copper meshes were cut into 3 cm × 2 cm (outer electrode) and 3 cm × 1 cm (inner electrode) rectangle sheets. Then, the rectangle sheets were gently rolled into cylindrical shape. Then, the inner electrodes were inserted into the outer electrode. Then, the two electrodes were fixed with a sealing cap holder, and were set in the tube. The two electrodes of the NEA system were connected to the TENG using electric wires and the TENG was simply driven by periodic human tapping motion. The

electrical output of the TENG were characterized using a low-noise electrometer (Keithley 6517B) with data acquisition system.

4.2.3 Fabrication of TENG

The TENG was composed of two crucial layers: a layer of Aluminum film and a very thin layer of PTFE film; the Al film function as both triboelectric layer and electrode while the PTFE film is merely triboelectric layer. The PTFE film was cleaned with ethanol and then carefully pasted on the layer of Cu electrode; make sure the PTFE film was well flattened to prevent any gas bubble. The aluminum electrode was glued to a sponge layer to form the top plate; the sponge helps to enhance the durability of the TENG system. All of the materials were wrapped and supported by Kapton tape. By the periodic pressing-releasing of the elastic top plate, alternating voltage and current could be generated between the Al film and Cu electrode. The NEA and TENG were connected using wires.

4.2.4 Nanowire Conductivity Measurement

Referring to previous report [118], the conductivity of the nanowires was measured using a semiconductor device analyzer (Agilent B1500A). In brief, after CuO nanowires were formed on the Cu mesh, the Cu mesh was sonicated in water so that the nanowires would drop off from the Cu mesh to form a suspension. Then the suspension was collected and drop-cast onto a silicon dioxide wafer, followed by patterning of the individual nanowire using electron-beam photolithograph setup and creating good contacts using thermal evaporation of Cr/Au (10 nm/190 nm). Then the

sample was ready to be measured with semiconductor device analyzer (Agilent B1500A).

4.2.5 Simulation of the Electric Field near a Nanowire

The electric field simulations were conducted using the commercial software package MatlabR2016a. The electric field in the vicinity of the CuO nanowire tip was simulated. The CuONW was attached to one of the two flat electrodes. Pores are perforated on the electrodes mimicking the experimental environment. The applied voltage between the two electrodes was 40 V, respectively. The density of the nanowire tip distribution was 10 / μm^2 and the nanowire diameter was 50 nm.

4.2.6 Cell Culture

The MiaPaCa-2 and K562 cells were respectively cultured in Dulbecco's Modified Eagle medium (Gibco) and RPMI medium. All the media were supplemented with 10% (v/v) fetal bovine serum (Gibco), 100 UI/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin to form the full cell culture medium. All the time, cells were cultured in an incubator with 5% CO_2 at 37 °C. When the cells reach full confluence, the cells were collected for the siRNA electro-transfection experiment.

4.2.7 Electro-transfection setup and process

The cells were harvested from petri dishes (use trypsinization method for MiaPaCa-2), followed by being centrifuged and re-suspended in fresh serum-free DMEM medium (for MiaPaCa-2) or RPMI medium (for K562); the cell density was altered to 2×10^6 cells/mL. Cy3 labeled siRNA (siRNACy3) was added into the cell

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suspensions to reach a concentration of 10 µg/mL, followed by injecting 200 µL siRNA loaded cell suspension into the chamber of the T-NEA system using a sterile syringe. The electro-transfection process simply starts by tapping the TENG periodically at a frequency about 3Hz for 10 to 40 secs. After treatment, the cells were collected from the chamber of the T-NEA with a sterile syringe and transferred to 6-well plates. The cells were cultured in incubator for another 12 hours before being analyzed.

4.2.8 Fluorescence imaging

The T-NEA treated cells were washed thrice with FBS-free DMEM media. Subsequently, the cells were seeded in six-well plate and continually culture for 12 hours. The in vitro fluorescence images were captured using two microscopes; an inverted epifluorescence microscope (Nikon, Eclipse-Ti) and a laser confocal scanning microscope (FV3000, OLYMPUS). For quantitative analysis, the cell numbers and overall fluorescence intensity were determined using a FACScalibur flow cytometer (Becton Dickinson, Mississauga, CA), in which Cy3 (filter set was PE) was applied as the luminescent marker to determine the transfection efficiency quantitatively. The transfection efficiency was calculated as the ratio of number of cells in Q4 to the total cells (Q1+Q2+Q3+Q4), indicating the percentage of cells that siRNA is successfully delivered. Then, the overall fluorescence intensity of each experimental group was compared statistically.

4.2.9 The analysis of the gene transcription level

The quantitative Real-Time PCR was employed to assess the transcription level of siRNA targeting gene following our previous experience⁴². After MiaPaCa-2 and

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K562 cells were electro-transfected with K-Ras and bcr-abl siRNA respectively using the T-NEA system, the cells were further cultured for 48h before being harvested. The transcription level of K-ras (MiaPaCa-2) and bcr-abl (K562) gene were investigated using the method described in previous work 38, 40. Briefly, the total RNA of the cells was extracted using TRIzol reagent (Invitrogen) and the total RNA concentration was determined using a micro-spectrophotometer (Nano-Drop ND-1000). By using reverse transcriptase kit (Promega), 2 µg of total RNA was reverse transcribed to cDNA, which was then amplified by Quantitative Real-Time PCR technique. All PCR experiments were completed using an ABI prism 7500 applied Biosystems system. The mRNA levels of the targeted genes were normalized based on the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers used were as follows: K-Ras: 5' -AGAGTGCCTTGACGATACAGC-3' (sense), 5' -ACAAAGAAAGCCCTCCCCAGT-3' (anti-sense); bcr-abl: 5' -CATTCCGCTGACCATCAATAAG-3' (sense); 5' -GATGCTACTGGCCGCTGAAG-3' (anti-sense), GAPDH: 5' -ACCACAGTCCATGCCATCAC-3' (sense), GAPDH 5' -TCCACCACCCTGTTGCTGTA-3' (anti-sense).

4.2.10 The assessment of the gene expression

The expression level of protein on the MiaPaCa-2 and K562 cells were evaluated by western-blotting as our previous report 43. Briefly, the siRNA-transfected cells by T-NEA system were seeded in six-well plates (1×10⁵ cells/well) for further culture of 48h. Prior to the isolation of protein, the cells were washed twice with PBS and harvested using lysis buffer (50mM Tris-HCl PH-7.4, 150mM NaCl, 1mM EDTA,

and 1% Triton X-100,). The total isolated protein concentration was quantified using a microspectrophotometer (Nano-Drop ND2000). 5 μ g of denatured cellular extracts were separated by 10% Tris-glycine SDS-polyacrylamide gels using the Mini-Sub Cell GT system (Bio-Rad, CA). Protein bands in the gel were then transferred to Nitrocellulose Blotting membranes. Membranes were incubated in block buffer for overnight at 4°C followed by washing with buffer (1 $\times$ PBS, 0.05% Tween 20) and 3% nonfat milk, and then incubated for 4 hours with the primary mouse anti-human K-ras antibody (ab55391, Abcam), or anti-human bcr-abl antibody (ab187831, Abcam) or anti-human GAPDH antibody (ab8245, Abcam). The membranes were washed with PBS/Tween-20, then HPRT (hypoxanthine guanine phosphoribosyl transferase)-labeled anti-rat secondary antibody was used for secondary incubation for one hour at room temperature. K-ras, bcr-abl and GAPDH proteins were then visualized with chemiluminescent substrate (ECL detection reagent, Sigma-Aldrich, USA) by the enhanced chemiluminescence system.

4.2.11 Cell proliferation and apoptosis

The cell viability and cell proliferation were evaluated by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 1×10^4 siRNA-transfected cells and 1×10^4 untreated cells were seeded in 96-well plates respectively for 48h post electro-transfection. 20 μ L of MTT solution at concentration of 5mg/ml was added to each well and then the cells were put back into incubator. 4 h later, the solution was discarded, followed by adding 100ul of DMSO and incubating for 15 mins in incubator. The solution turns purple at the end of the treatment. The number of viable cells is proportional to the absorbance of the solution at 490 nm, and the absorbance was measured on an automated plate reader. The cell viability was

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calculated as the absorbance ratio of the electro-transfected cells over the negative control group, which is the untreated cells. The apoptosis rate of the electro-transfected cells was determined using a FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen™) following the manufacturer's recommendations. In short, approximately 1×10^6 treated cells were seeded in a six-well plate for culture of 72 hours. The cells were then collected and washed twice with cold PBS and suspended in binding buffer. Then, flow cytometry analysis was used to determine the apoptotic cells.

4.3 Design of the NEA system

The structural configuration and photograph of NEA device are shown in **Figure 4.1a**. The NEA device constitutes of two mesh electrodes based on copper oxide nanowires (CuONW) array. Two toroidal parallel CuONW mesh electrodes were installed inside a tube, were fixed by sealing cap holder (**Figure 4.1b**). The CuONW mesh electrodes was fabricated as described in the experimental and method part [118]. Briefly, the nanowires mesh electrode was fabricated by a simple one-step process of oxidizing Cu mesh at 500°C for 4 hours. The color of Cu mesh changes from coppery to black due to the formation of CuO nanowires. The copper mesh has a 500×200μm pore size which is larger than the size of the cells (~10μm), making sure the cells can pass through freely and still ensures a high probability of cell electroporation. As the cell suspension was squeezed in the narrow gap between electrodes in the NEA device, more cells were brought close to the nanowire electrode with a larger exposed cell membrane surface. The surface morphology of the mesh electrode was characterized using a scanning electron microscope (SEM) to confirm that the nanowire arrays were formed. The smooth and flat surface of Cu mesh became nanowire structure after the oxidizing process, as presented in **Figure 4.1b inset** (lateral view). The density of nanowire is about 10/μm². Typical nanowire on the mesh surface has about 5 μm length. The nanowires were grown rooted to the mesh surface with diameters mostly below 50 nm. During operation, the static electricity was applied to the nanowires of the two electrodes. According to previous reports [118], the structure of nanowire was expected to amplify the electric field for enhancing the electro-transfection efficiency. The TENG was connected to NEA devices using electric wires. The whole experimental setup of the T-NEA system is shown in **Figure 4.1b (4)**.

Figure 4.1 Schematic illustration and real images of the T-NEA experimental setup (a) Schematic illustration of the experimental setup. The NEA were connected to TENG with two electric wires. The siRNAs were electro-transfected into suspended spherical cells by T-NEA system (b) (1-2) Photograph of Cu mesh and processed Cu meshes. SEM image of surface on the Cu mesh and oxidized Cu mesh (inset). The vertical nanowires on the plate electrode were formed on the surface of Cu mesh at 500°C for 4 hours. Typically, the nanowire has a length of 5 μ m and the nanowires array density is about 10 nanowires/ μ m². (3) The inner and outer electrodes were fabricated by wrapping the processed CuO mesh. (4) The photographs of the TENG-driven NEA system.

4.4 Electrical Performance of TENG

Due to its lightweight, low cost and decent strength, PMMA was selected as the substrate material. The two poly (methyl methacrylate) (PMMA) panels were pasted and wrapped into the elastoplastic. On the top side, a 5mm thick sponge layer as stress buffer was bound to the PMMA substrate, which can increase the durability of the TENG. Next, an aluminum film layer was pasted on the sponge layer in sequence. The contact electrode on the other side, a layer of copper film was laminated between a layer of poly(tetrafluoroethylene) (PTFE) film (electrification material) and the PMMA substrate. The photograph of the TENG fixed in a bigger PMMA board is presented in **Figure 4.1b (4)**.

Some previous studies showed that the TENG can be triggered by winds, heart beats, waves and vehicle wheels [121]. In this project, the TENG are driven by human motions. By periodic pressing-releasing motions of the top and bottom plates that are parallel to each other, the open-circuit voltage and short-circuit current of TENG were generated. The TENG gave transferred charge during on circuit was around 90nC (**Figure 4.2a**); a pressing-induced voltage of about 160V (**Figure 4.2b**); the short-circuit current reached a peak value of +15 and -5 μ A. Because the separation of the contact electrode and PTFE is supported by the restoring force of the elastoplastic, which maintain a fixed distance between them, a stable electric output can be provided by TENG without any addition of circuit to change the output current. The basic operating principle of TENG is described in Section 5.3. Briefly, when the top and bottom plates are separated in the initial state, no electrical potential difference is detected because of the absence of contact-induced charge transfer. Upon pressing of TENG, the aluminum sheet film touches the PTFE film, injecting electrons from Al to the PTFE film due to the contact electrification to build potential difference between the two materials. Then, the surfaces of the Al and PTFE layers are positively and negatively charged, respectively. Thus, the potential difference causes contact induced charges to transfer between the Cu electrodes on the two sides, and AC (alternate current) was generated. Therefore, the TENG can serve as a self-powered electrical stimulator that generates alternating voltage for efficient cell electroporation.

Figure 4.2 Electrical Performance of TENG. The induced transferred charge(a) and voltage(b) from TENG under periodic external human motions force. (c) Conductivity measurement of a single nanowires. (d)The simulation of electric field distribution with an applied voltage of 40 V of vicinity of the tip structure of CuO nanowires (diameter, 100 nm; length, 5 μm) in cell culture medium showing the enhancement of the electric field strength (the scale is 1 μm).

Like what occurs in many proof-of-concepts for micro/nano cell electroporation device [105, 108, 109, 111, 118, 120], nanowires in NEA amplify the electric field with their nanoscale far end that protrudes towards the cell. According to the theory of electric field distribution, the enhanced electric field strength depends on the diameter and length of nanowire[105]. The conductivity of nanowires is essential in building the electric field, so the nanowires should have higher conductivity than the cell culture medium in order to amplify electric field. As shown in **Figure 4.2c**, the conductivity of nanowires was determined by nanowire electrical transport measurement by following the Cui's method [118]. The nanowire had conductivities of about 300S/cm.

Comparing to DMEM (approximate conductivity: 1.43S/cm) [122], nanowires are conductive enough to build a strong electric field in cell culture medium near the

nanowires. As demonstrated in electric field simulation (**Figure 4.2d**), the transmembrane potential near nanopillar on a suspended spherical cell is above 4×10^7 V/m for electroporation. Electroporation for transfecting siRNA into mammalian cells, the electric field larger than $10^3 \sim 10^6$ V/m is required [104]. Therefore, the NEA device can theoretically provide the enough strength of electric field near the tip structure to achieve cell electroporation with a much lower applied voltage providing by TENG. The sharp tip structure of NEA amplifies the electric field in the vicinity of the nanowires, hence yielding a great performance on siRNA transfection in this study. As the amplified electric pulses affect mainly a tiny portion of the cell membrane each nanopillar faces, this gives localized electroporation benefit the subjected cell viability. Furthermore, unlike microfluidic electroporation [112, 113], and the NEA doesn't need fluidic components for trapping cells to realize siRNA electro-transfection. Hence, the operation procedure of T-NEA system is therefore more convenient, and similar simplicity procedure to the commercial electroporation systems.

4.5 Gene delivery to Cells

We first performed NEA electroporation on MiaPaCa-2 cells (pancreatic cancer cell line) and K562 cells (leukemia cancer cell line) for siRNA delivery to demonstrate its effectiveness for both adherent and non-adherent cancer cell lines, in which the latter K562 cell was well-known as hard-transfected cells [123]. The used siRNA was labeled by Cy3 fluorescence dye for visualization of siRNA delivery. The harvested 200 μL cell solution at 1×10^5 cell/mL concentration was injected into NEA chamber by sterile syringe. As electro-transfection time increased from 10 secs to 40 secs under the same given electric pulse frequency (single pulse with a duration of 10ms) in T-NEA system, the intracellular red fluorescence gradually became brighter in both treated MiaPaCa-2 (**Figure 4.3a**) and K562 cells. The more intense fluorescence means higher siRNA amount inside cells. Quantitative values regarding transfection in terms of the Cy3-fluorescence signal strength were obtained by flow cytometry assessments (**Figure 4.3b**). The siRNA electroporation efficiency and the fluorescence intensity were obviously elevated as the electro-transfected time increased in T-NEA system (**Figure 4.3c and 4.3d**).

When the process time is increased to 40 seconds, it seems to get saturated electroporation efficiency. Noteworthy, under same electro-transfection time, the transfection efficiency on the MiaPaCa-2 cells are much higher than that of K-562 cells (**Figure 4.3d**). These results are attributed to the size of cells affect siRNA transfection efficiency in T-NEA system, which determines the number of nanowires cell facing during electro-transfection. The induced transient pores and the overall effective permeable area on the cell membrane varies with the size of individual cells. In other words, a big cell faces more nanowires and should have more induced transient pores to facilitate cellular siRNA uptake. The different sizes of MiaPaCa-2 cells (about 10 nm) and K-562 cells (about 5 nm) determine the transfection siRNA amount in the same applied electric field. It also was conceivable that delivery of siRNA to K562 cells was also limited due to their higher cell density (high nucleo-cytoplasmic ratio under rapid proliferation) as compared to MiaPaCa-2 cells [124]. By increasing the numbers of applied pulses, the transfection efficiency of siRNA was also improved for MiaPaCa-2 and K562 cells, implying the T-NEA can be used to deliver the desired amount of siRNA into cells in a controllable manner.

Figure 4.3 Evaluation of electro-transfection efficiency using cy3 labelled siRNA. (a) The post electro-transfected MiaPaCa-2 cells were observed under confocal laser scanning microscopy (CLSM). MiaPaCa-2 cells were electro-transfected by T-NEA system for different time at the same pulse frequency, and then the cells were seeded in the cell culture dish for 6 hours to adhere the bottom of cell culture dish. The nuclei were stained with DAPI (Blue), and the siRNA was labeled with Cy3(Red). (b) Evaluation of transfection efficiency of siRNA in the MiaPaCa-2 cells were detected by flow cytometry. (c) Statistical calculation of transfection efficiency of siRNA in MiaPaCa-2 (Fig. 2b) and K562 cells (Fig. S7b). The ratio of cells in Q4 to the total cells (Q1+Q2+Q3+Q4) yielded the transfection efficiency of siRNA. (d) The average fluorescence intensities resulting from Figure 2b and S7b, depicting the performance of T-NEA as an outstanding electroporation system. Results are represented as means $\pm$ SD, n = 3.

4.6 Specifically Silencing the Expression of K-ras Gene on MiaPaCa-2 Cells and bcr-abl Gene on K-562 Cells

To further demonstrate the effectiveness of T-NEA on siRNA transfections, we elaborately selected siRNAs with designed sequences that could specifically silence the expression of K-ras gene on MiaPaCa-2 cells or *bcr-abl* gene on K-562 cells. Meanwhile, the NC-siRNA (not silence any targeting genes) were introduced as control groups. The gene knockdown efficiency of K-ras and *bcr-abl* were investigated by Real-time PCR and Western-blot, respectively. As shown in **Figure 4.4**, clear transcriptional suppression of targeting gene expression on MiaPaCa-2 and K562 were observed after the corresponding siRNA were transfected into the cells by T-NEA device. The higher the TENG given electric pulse, the higher the inhibition efficiency on gene expression. In 40 second-applied pulse treated groups, the K-ras gene transcription on the MiaPaCa-2 cells has been significantly inhibited ($46 \pm 6.29\%$) comparing with these of the un-treated treated groups (**Figure 4.4a**). Similar knockdown enhancement of *bcr-abl* gene transcription was also observed in K562 cells ($31.6 \pm 6.67\%$ in 40 second-applied pulse treated groups), confirming siRNA transfection effectiveness of T-NEA on non-adherent cells as well (**Figure 4.4b**). The gene expression on the protein level were also evaluated by Western-blot. After siRNA transfected with T-NEA, the targeted protein expressions were effectively repressed on MiaPaCa-2 cells and K562 cells (**Figure 4.4c and 4.4d**), which is consistent with the inhibition on gene transcription level. It is worth pointing out that the expression of GAPDH was not affected by the applied pulse, which means the T-NEA does not disrupt the expression pattern of siRNA non-targeting gene, conforming its low non-targeting effect in the molecular level.

Figure 4.4 Gene transcription and expression level on MiaPaCa-2 and K562 cells. After electro-transfected siRNA by T-NEA system on MiaPaCa-2 and K562 cells, the treated cells were continually incubated for 48 hours. After that, K-ras gene transcription level (a) and expression level (c) on the MiaPaCa-2 cells were evaluated by RT-PCR and Western-blot. Similarly, after electro-transfected bcr-abl siRNA by T-NEA system for 48 hours, the bcr-abl gene transcription level (b) and expression level (d) on the K562 cells. Error bars indicate the SD, *P < 0.01 compared with the un-treated group, n=5.

Figure 4.5 After electro-transfected designed siRNA into cells using T-NEA system, the relative bioactivity of cells was effectively regulated. (a,b) Post-transfected with the T-NEA system, the cell proliferation were assessed by MTT assay.(c) The cell apoptosis was investigated on the MiaPaCa-2 cells by FITC Annexin V and Propidium Iodide (PI) co-stained apoptosis assay. (d) Late apoptotic counts of MiaPaCa-2 and K562 cells in the upper right quadrant (Q2) of different time treatment groups. Error bars indicate the SD, *P < 0.01 compared with the un-treated group, n=5.

RNA interference (RNAi) targeting the K-ras oncogene mutation in pancreatic cancer mediated by siRNA transfection is a very promising treatment. Reports have shown that down regulated K-ras gene expression causes reduced proliferation and increased apoptosis in MiaPaCa-2 cells [125]. Leukemia is mostly caused by chromosomal translocations between chromosomes 9 and 22 that result in an oncogenic hybrid *bcr-abl* fusion gene. The *bcr-abl* chimeric protein is known for its increased active tyrosine kinase activity, which leads to oncogenic growth and reduction of

apoptosis, thus promoting chronic myelogenous leukemia [123]. According to our finding of high gene knockdown efficiency by T-NEA, we then examined the anti-proliferation effect on MiaPaCa-2 cells and K-562 cells using MTT assay. As shown in **Figure 4.5a and 4.5b**, after incubation for 48h, the cell proliferation of MiaPaCa-2 cells and K-562 cells was effectively inhibited. The inhibition efficiency is in proportion to the given pulse times. Meanwhile, the cell apoptosis was investigated by FITC Annexin V and Propidium Iodide (PI) co-stained apoptosis assay on treated MiaPaCa-2 cells (**Figure 4.5c**) and K562 cells. These results indicated the cell apoptosis was induced when the therapeutic siRNAs (K-ras siRNA or *bcr-abl* siRNA) was delivered into the targeted cells by T-NEA, achieving the functional regulation by silencing targeted gene in the MiaPaC-2 and K562 cells. The more electroporation duration time was given on the cells, the more cell apoptotic numbers were promoted (**Figure 4.5d**). The cell proliferation and apoptosis were not affected when the NC-siRNA (negative control siRNA) was electro transfected into cells by T-NEA system, further indicating the biosafety of T-NEA as siRNA electro-transfection system.

More cell lines were tested to demonstrate the universality of T-NEA as siRNA electro-transfection system. Similar high transfection rates in varies cell lines are observed under the same electric pulse conditions. More importantly, the cell electro-transfection with un-processed Cu plate electrodes didn't present any intracellular red fluorescence (**Figure 4.6a**). These phenomena confirm that the performance of NEA on siRNA transfection is indeed attributed to the nanowire features on the electrode surface. Only incubating the cells with the siRNA^{Cy3} in the NEA chamber that no TENG supplies the driven-power, there is no any significant red color were observed inside cells (**Figure 4.6b**). This result clearly indicates the importance of TENG in the electro-transfection system. The electric current could induce thermal damage and hydrolysis-

associated negative impact on treated cells, which caused an extra loss on the overall cell survival rate.

It is well known that efficient delivery of siRNA into cells requires not only high intracellular accumulation, but also efficient transportation into the cytoplasm where siRNA recognizes and binds to RNA to silence the target gene. Endosomal escape is one of the major barriers for non-viral siRNA delivery due to siRNA trapped in endosomes often trafficked into the lysosome where siRNA is degraded by hydrolysis enzymes. The electroporation overcomes this bottleneck by forming pores in the cell membrane, by which the siRNA straightly passes through the cell membrane into the cytoplasm. Consequently, these contacting areas of nanowires and cell membranes facilitate to form temporary openings pores for siRNA electro-transfection. Results from T-NEA system show high siRNA transfection into pancreatic cancer cells, as well as leukemia cells with high biocompatibility, which apparently attributes to the high voltage, low current, and nanowire structure of the T-NEA system. This property of T-NEA system overwhelms the commercialized bulk electroporation device in order to gain the expected transfection efficiency often at the expense of the cell viability. The versatility, efficiency, and uniformity of the T-NEA system serve as a powerful and reliable platform for high-throughput intracellular gene delivery and transfection.

Figure 4.6 Negative control groups for siRNA delivery experiment (a) The MiaPaCa-2 cells were electro-transfected with un-processed Cu net electrodes for 40 secs powered by TENG, in which there are no nanowires on the surface of electrodes. The blue fluorescence indicating the DAPI-stained cell nuclei. (b) The MiaPaCa-2 cells were incubated in the T-NEA system for 40 secs without power support from TENG. No significant red fluorescence from Cy3-labeled siRNA was observed inside cells.

4.7 Conclusion

In this study, the CuONW mesh was fabricated using thermal oxidation. The formation of nanowires was characterized and confirmed by SEM observation. The NEA device was constituted with two parallel CuONW mesh electrodes. The power source was supplied by TENG. Depending on the electric pulse supplied by TENG and amplified electric field effect by nanowires, the cell membrane opens transient permeability to siRNAs. Thus, the siRNAs were effectively electro-transfected into different kinds of cells (adherent and non-adherent). After therapeutic siRNAs were electro-transfected into cells by T-NEA system, the bioactivity of cells was regulated in relation to the targeted gene silencing. More importantly, the T-NEA system didn't produce any significant cell cytotoxicity as a result of the power characters of high voltage and low current supplied by TENG. Overall, we demonstrate a novel smart gene electroporation device, which is feasible and deserves further exploration in mRNA delivery, pDNA delivery, CAR-T and CRISPR/CAS9 gene editing system.

Chapter 5: Biochip for Monitoring of Trans-Epithelial Electrical Resistance of Gastrointestinal tissues

5.1 Introduction

To determine the viability of tissue samples is always challenging. The commonly used method, tissue sectioning, involves staining the tissue with biochemical dyes followed by cutting them into thin slices for observation under a microscope. This method is time-consuming and expensive. More importantly, the tested samples are disrupted due to the treatment so that they cannot be further used for experiments. Using electrical method provides an alternative strategy to determine the tissue viability. Trans-epithelial electrical resistance (TEER) method is a widely accepted quantitative technique to measure the barrier integrity of tight junction in epithelial cells. It is conducted by applying an AC electrical signal across a cellular monolayer, and then measuring voltage and current to calculate the electrical resistance of the barrier. The TEER measurement for monolayer cell culture has been well studied and there have been studies correlating the TEER values to the viability of the epithelial monolayer. This is because when epithelial cells become less viable, the tight junction between them is disrupted, resulting in an altered TEER value. Therefore, monitoring TEER might be an excellent electrical method for assessing cell viability because it is fast responsive, low-cost, label-free and most importantly, non-destructive to the testing samples. However, unlike the monolayer culture of cells, TEER measurements on tissue samples remain technically challenging due to some practical challenges.

In this chapter, biopsied tissues from porcine esophagus were first processed and only the part of interest (epithelial layer) for TEER measurements was extracted. These samples were cultured in three different media and their viabilities were assessed

Chapter 5: Biochip for Monitoring of TEER of Gastrointestinal tissues

periodically with a standard colorimetric method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The TEER of the tissues was measured using a homemade ring-shaped sample holder at different culturing days. The results indicated that the TEER values reflected the tissues viability changes. Additionally, a LOC device was designed and fabricated with dual functions of tissue culturing as well as real time monitoring the TEER on-chip. The biochip consists of four layers: the top and bottom PMMA layers with perfusion channels for perfusing culture medium, two middle PDMS layers with a central cavity as tissue sample holder. Ag/AgCl electrodes were inserted to connect to the top and bottom perfusion channels for TEER measurement. Live/dead staining and histological analysis were carried out to study the viability of the tissues cultured on chip. Furthermore, a fluorescent flux tracer was used to study the permeability of the cultured tissues and the results were compared with the changes in TEER values.

5.2 Materials and methods

5.2.1 Preparation of epithelial tissues from porcine esophagus

Cut the esophagus into several portions (about 10cm long) using a scalpel. Wash with Phosphate-buffered saline (PBS) a few times until the solution remains clear after washing. Strip off the muscular layer by tearing it apart with tweezers. Then the sample was kept in Leibovitz 15 medium with 10 U/ml dispase at 4degrees for 12 hours. The dispase will digest the bonding between mucosal and subcostal layer without harming the integrity of the mucosal layer. Thus, the mucosal layer can be carefully peeled off from the submucosal layer.

5.2.2 Protocols of tissue culturing in petri dish

After the tissue preparation steps, the samples were cut into 2cm×2cm pieces and divided into three groups. Each group was cultured with one culture medium respectively (D medium, R medium or K medium, the recipes are listed in the table below). All the samples were transferred into 6-well plates. Each well add 2 ml of culture medium. The tissue samples were then kept in an incubator at 37°C and 5% CO₂ for preserving their viability. In order to study the effect of media changing frequency on the tissue viability, samples were divided into three groups, changing medium at 12h, 24, and 48h intervals respectively.

Content	D medium	R medium	K medium
Base Medium	DMEM	RPMI	Keratinocyte Growth media
FBS	10%	10%	-
Insulin	1 ml/L	1 ml/L	-
Hydrocortisone	10 µg/ml	10 µg/ml	-
Epithelial Growth factor (EGF)	10 ng/ml	-	-
Transferrin	5 µg/ml	-	-
1-Thioglycerol	-	10 µM	-
HEPES	-	20 µM	-

5.2.3 Performing off-chip TEER measurement using homemade ring-shaped sample holder

Before start the experiment, all the tools (ring plates, magnets, tweezers, cutting board and Endohm-6) were rinsed with 70% ethanol followed by UV exposure for 1 h for sterilization. Take out one piece of cultured tissue from pertri dish and place it on the cutting board. Using a new disposable 6mm biopsy punch, cut out a 6mm disc from the tissue sample. Using a pair of tweezers, place the disc-shaped sample between the two PDMS ring plates. Then secure the sample and rings with two neodymium ring magnets, forming a complete sample holder. Fill the Endohm-6 with 200 μ l of DMEM medium. Fill the bottom side of the neodymium magnet with DMEM (50 μ l), ensuring no air bubbles are trapped inside the column. Place the sample holder into the Endohm-6, with the side filled with DMEM medium facing down. Fill 100 μ l DMEM at the central hole. Close the cap and adjust the position of the electrode on the cap to make sure it is touching the medium in the central hole. Thus, the TEER value will be shown on EVOM-2. After recording the TEER value, take out the tissue sample from the sample holder and transfer it into a 96 well plate for the MTT assay subsequently.

5.2.4 MTT assay and live/dead staining

MTT assay: MTT solution is prepared by dissolve MTT power into PBS solution, making a final working solution with a concentration of 5mg/ml. The working solution appears yellow. After taking TEER measurement, transfer the 6mm disc-shaped tissues into a 96-well plate. Adding 150ul of culture media, followed by adding 50ul of MTT working solution. Transfer the 96-well plate into an incubator at 37C and 5% CO₂. Incubate for 8h. After 8h, the white tissue samples become purple/black. Remove all the solution in the 96-well plate, followed by adding 150ul of DMSO

solvent. Incubate for 1h. Remove all the solid tissue sample, keep the DMSO solution which is already turned purple. Measure the absorbance at 540nm in a plate reader.

Live/dead staining: Prepare the assay solution by adding Calcein AM and Ethidium homodimer-1 into PBS. (Calcein AM conc. = 2uM; Ethidium homodimer-1 conc. = 4uM) Since the assay solution is only stable for a few hours, fresh assay solution need to be prepared before each assay. Transfer the 6mm disc-shaped tissues into 96-well plate. Wash with PBS. Add 100ul of assay solution into each well. Keep out of light for 30 to 60mins. Wash with PBS two times to remove the background fluorescence. Check the fluorescence using a fluorescence microscope. For viewing Calcein AM (live cells), use blue excitation light and green filter for collecting the fluorescence light. For viewing Ethidium homodimer-1 (dead cells), use green excitation light and red filter for collecting the fluorescence light.

5.3 Peel off the epithelial layer from the submucosal layer

As shown in the sectioning image in **Figure 5.1a**, esophagus consists of three layers; mucosa, submucosa and muscular layer. Mucosa layer is the part of interest for the following experiments. The muscular layer can be simply stripped off from the other two layers by tearing them apart. The challenging part is how to separate the mucosa layer from the rest because the mucosa and submucosa layer are closely connected by connective tissue. In order to peel off the mucosa layer, the sample was stretched and pinned, using bobby pins, onto a Petri dish coated with a thick layer of PDMS (**Figure 5.1c**). Then a pair of micro-scissors was used to separate the mucosa from the submucosa layer under a dissection microscope. Although this method works, it is too time-consuming and the tissue integrity might be disturbed during the cutting process. So another chemical peeling off method was explored instead. The samples were first immersed in an enzyme solution and this enzyme, dispase, digests only the bonding in connective tissues without harming the integrity of the mucosal layer. Thus, the mucosal layer can be carefully peeled off from the submucosal layer and transferred into petri dish for culturing (**Figure 5.1d**). The dried thickness of the peeled mucosa was measured to be 0.08 mm, while the thickness was 0.6 mm before peeling.

Figure 5.1 Peel off epithelial tissue from porcine esophagus. (a) Structure of esophagus tissue. (b) Porcine esophagus (c) Biopsy sample pinned in a glass dish coated with a thick layer PDMS. (d) The epithelial layer obtained after enzyme treatment.

5.4 Mechanism and method of measuring TEER of monolayer cells

A predominant TEER method is to culture cells on permeable membranes in a specially designed dish (Transwell dish) and then measure the TEER with a pair of chopsticks-shaped electrodes. The mechanism is illustrated in **Figure 5.2**. The Transwell dish consists of an inner well and an outer well. The bottom of the inner well is a porous membrane which is permeable to water and ions, but not to cells. The cells plated on the membrane will grow and spread over the membrane until the membrane is fully covered. Thus, the cells will form a barrier layer. Then a pair of chopstick electrodes can be put in to measure the TEER. One leg of the electrodes sits in the inner well while the other leg in the outer well to form a closed circuit. The measured resistance values are then normalized by multiplying the area of the membrane, resulting in a unit of [$\Omega \cdot \text{cm}^2$]. It is equivalent to resistivity $\times$ length, which should be constant when the area is fixed.

Figure 5.2 The mechanism of TEER measurement and image of Transwell dish

5.5 Challenges in TEER measurement of tissues and a suggested solution

Unlike measuring TEER of monolayer cells, measuring tissue samples is much more challenging. Basically, there are two major challenges that make the conventional Transwell method no longer applicable. The first challenge is, unlike cells which are adherent on the porous membrane, tissue samples are floating in the medium. Thus, the sample cannot form a barrier layer against medium and the two electrodes become short-circuit. The second challenge is, unlike cells which are spreading to cover the entire porous membrane, tissue samples are not outgrowing. As the sketch in **Figure 5.3a**, the size of the sample is smaller than the diameter of the porous membrane and the shape is irregular. In such a case, the non-covered area of the porous membrane will provide an easier electric pathway which will effectively shunt the pathway through the tissue.

To solve the problems, we come out with a solution as sketched in **Figure 5.3b**. Two ring plates with a central hole are used, between which the tissue is sandwiched. The size of the tissue is larger than the central hole. Thus, there will be no electrical leakage from the non-covered area of the porous membrane and the tissue will not fold up nor float around. Before the TEER measurement, a piece of tissue sample was placed on a piece of porous membrane, followed by being cut into 6 mm disc-shaped with a biopsy punch as shown in **Figure 5.4**. **Figure 5.5** shows the experimental setup for the TEER measurement. The two ring plates (**Figure 5.5a**) were homemade using PDMS from 3D printed mold. There is a 6mm circular groove on the ring plate to accommodate tissue samples and the diameter of the central hole is 4mm. Once the tissue sample was loaded (**Figure 5.5b**), two pieces of magnets were used to seal the PDMS ring plates (**Figure 5.5c**) to form a complete sample holder. Then the entire holder was put into a

special chamber (Endohm-6) for TEER measurement (**Figure 5.5d and 5.5e**). The Endohm-6 chamber is specially designed for TEER measurement with one electrode at the bottom of the chamber and the other electrode on the cap. Before taking measurement, 200 μ l of medium was added into Endohm-6 chamber and 100 μ l of medium was added into the central hole of the top magnet. Then the position of the electrode on the cap was adjusted to just touch the medium in the central hole. Thus, electrical circuit was formed between the two electrodes through the tissue sample, and TEER values can be measured now.

Figure 5.3 Challenge in measuring TEER of tissue and the proposed solution. (a) A challenge in measuring TEER of a tissue. (b) The proposed solution to overcome the challenges.

Figure 5.4 Preparation of 6 mm disc-shaped tissue sample for TEER measurement

Figure 5.5 Experimental setup for the TEER measurement

5.6 Tissue TEER and MTT-based viability vs the number of culturing days (off-chip culturing and measurement)

The tissue samples are cultured in three media, abbreviated as K medium, R medium and D medium. For every two days, TEER was measured using the method described in the above section. Then the measured tissue was transferred into a 96-well plate for assessing tissue viability using MTT assay. Briefly, the intracellular enzyme may convert MTT into insoluble formazan, which is then absorbing wavelength at 540 nm in dimethyl sulfoxide (DMSO) solvent. Since MTT solution is highly stable, the tissue sample can be immersed in MTT solution for a prolonged period to make sure the MTT solution can diffuse to the inner part of the tissue. After 8h incubation, the yellow MTT solution becomes purple. Then the 96-well plate was put in a plate reader to measure the light absorption at 540nm. The absorbance values are proportional to the number of viable cells. The tissue viability is calculated as the ratio of absorbance values over the original absorbance value on day 0.

The TEER values of tissues cultured in three different media are plotted in **Figure 5.6a**, and K medium exhibits the lowest drop in TEER values, indicating the best ability in preserving the tissue viability. In **Figure 5.6b**, the MTT-based viability and normalized TEER vs the number of culturing days is plotted. Same trends are observed in the TEER values and tissue viability. Both decrease as the number of culturing days increases. Very good correspondence is obtained, indicating that TEER values can be used as a good indicator of the overall tissue viability.

Figure 5.6 The TEER values of tissues cultured in petri dish. (a) The TEER values of tissues cultured in three different media. (b) The normalized TEER and tissue viability assessed using MTT method.

5.7 Design and fabrication of the biochip

Culturing tissue biopsies in a microfluidic device will provide a more holistic model as compared to culturing the sample in the static medium in petri dish, because the microfluidic device can provide a more reproducible environment which is more similar to *in vivo* conditions. Therefore, a biochip was developed for preserving the viability of tissue samples, as well as integrated with electrodes for on-chip TEER measurement. Thus, the complexity of measuring TEER could be much simplified and real-time monitoring of TEER could be achieved.

As compared to biochip for cell culturing, the challenge of culturing tissue on-chip is how to load the tissue sample. For cell culturing, a fully sealed chip can be fabricated then the cells are loaded through the inlet as cell suspension. However, this sample loading method is not feasible for tissue samples because they are solid pieces. Hence, the chip needs to be somehow dismountable so that the tissue sample can be loaded. Adhering to this design concept, a biochip consisting of four layers was designed and shown in **Figure 5.7**. The design consists of 4 layers: the top and bottom layers are made of PMMA with perfusion channels; the middle two layers are made of PDMS with a central hole where the tissue sample will be placed and “sandwiched”. The four layers are stacked together and secured firmly by screws. Four Ag/AgCl wires are inserted into the perfusion channels, two connecting to the top channel while the other two connecting to the bottom channel, functioning as the electrodes for TEER measurement. **Figure 5.8a** are the images of the fabricated four layers and **Figure 5.8b** is the image of the assembled biochip. During TEER measurement, the four electrodes are connected to the TEER measuring meter through a homemade connection adaptor.

Figure 5.7 Sketch illustration of the biochip design. The design consists of 4 layers: the top and bottom layers are made of PMMA with perfusion channels; the middle two layers are made of PDMS with a central hole where the tissue sample will be placed and “sandwiched”.

Figure 5.8 Images of the as-fabricated biochip and the experimental setup for TEER measurement.

5.8 On-chip TEER monitoring and tissue viability assessment by histological analysis and live/dead staining

Once the disc-shaped tissue sample was mounted on the biochip, the screws were firmly tightened to make sure the chip was fully sealed. Then the culture medium was continuously perfused into the chip driven by syringe pumps. The TEER values were recorded every 12h for each chip and the results are plotted in **Figure 5.9**.

In the previous section, the tissue viability is quantitatively assessed using MTT assay method. However, MTT method is designed for assessing the viability of monolayer cell culture. Using this method to determine the viability of the tissue sample may not be very accurate. Therefore, other viability assessing method is needed to verify the reliability of the MTT results. Histology analysis is the most reliable method. At different intervals, the tissue samples cultured on-chip were taken out and fixed in formalin solution, followed by dehydration. Then the sample was embedded in wax to be cut into slice of 5um thickness, followed by hematoxylin and eosin staining. After the treatment, the tissue sample can be observed under a microscope to determine the viability of the sample. The histology images are also shown in **Figure 5.9**. Hematoxylin stains nuclei of cells dark blue/purple, while eosin colors cytoplasm structures in various shades of red, pink, and orange. The histological images are in good agreement with TEER values. On day 0, the sample is most fresh with highest TEER value. The image is very sharp in color change; each part of the tissue is highly distinguishable. On day 4, the TEER is halved. The color of the histology image becomes more uniform; the individual part of the tissue becomes less distinguishable. On day 8, the TEER is only about 15% of the original value on day 0. The histology image shows that there are large blank areas inside the tissue; the part of cells are dead and gone. The dark blue/purple cell nuclei also become less visible.

Figure 5.9 TEER of tissues culture on-chip and images after histological staining

Live/dead assay is another commonly used technique to assess the viability of monolayer cell cultures. The assay solution contains two chemicals (Calcein AM and Ethidium homodimer-1) which can stain live and dead cells respectively. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the nonfluorescent cell-permeant calcein AM to calcein, which exhibits strong green fluorescence. Ethidium homodimer-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells. Although live/dead assay is also designed for assessing monolayer cell cultures as MTT method, this method was still explored in the experiment because live/dead assay involves of fluorescence imaging which allows the cultured tissues to be assessed directly on-chip; no need to be removed from the biochip. **Figure 5.10** shows the

fluorescence images of the tissue samples cultured on-chip for 12 h and 96 h respectively. For 12 h sample, the green fluorescence in the image is highly uniform and red fluorescence is only found at the edges of the tissue, indicating a healthy status of the sample. On the other hand, after 96 h culturing on-chip, the image shows dispersed green dots and red dots can be found everywhere in the image, indicating that the tissue surface is no longer uniform and there are dead cells everywhere in the sample.

Figure 5.10 The fluorescence images of the tissues cultured on-chip after being stained by live/dead assay solution.

5.9 Tissue permeability and TEER

To quantify the permeability of the tissues, a fluorescent flux tracer, fluorescein isothiocyanate (FITC) -dextran, was used. As sketched in **Figure 5.11a**, medium with 5 $\mu\text{g/mL}$ FITC-dextran was perfused from the top channel and the effluent from the bottom channel was collected for fluorescence intensity measurement. Excitation wavelength = 480nm, emission wavelength = 530nm. A baseline fluorescence intensity was obtained by separating the top and bottom channel with an impermeable PTFE film and measuring the fluorescence intensity of the bottom effluent. For the fluorescence intensity obtained in the case of tissue samples, the baseline value is deducted. The intensity top effluent is about 45000 A.U. The fluorescence intensity of the bottom effluent is plotted in **Figure 5.11b** and this can be used as an indicator of tissue permeability. The normalized TEER and normalized permeability are plotted in **Figure 5.11c**. It is observed that TEER is pseudo-complementary to tissue permeability.

Figure 5.11 Experiment to correlate the tissue TEER with its permeability. (a) illustration of how the tissue permeability is quantified. (b) The fluorescence intensity of the bottom effluent, which is a indicator of the tissue permeability. (c) The normalized TEER and normalized permeability.

5.10 Conclusion

In this chapter, TEER was demonstrated to be an excellent method to assess tissue viability because it is easier, faster, label-free & non-destructive, and we proved it was correlated with the viability of tissue determined using MTT, histology and live/dead staining method. Besides, a dual function biochip is developed for culturing epithelial tissues as well as measuring the tissue TEER on-chip. The use of the biochip could significantly reduce the complexity of measuring TEER and make real-time TEER monitoring possible. The biochip is fabricated in lost-cost processes and more importantly, the biochip is dismountable so that it can be reused after cleaning.

Chapter 6: Triboelectrification-enabled detection of heavy metals in water

6.1 Introduction

Most heavy metals (for example, Pb, Cd, As) are deleterious even at very low concentrations because they have high tendency to form complexes with biomolecules containing nitrogen, sulfur, and oxygen, resulting in many serious consequences such as structural changes in proteins, breaking of hydrogen bonds and inhibition of enzymes. Furthermore, they may induce oxidative stress that would produce free radicals, such as reactive oxygen species (ROS), possibly leading to alterations in the cell signaling pathway. Heavy metals are not biodegradable and therefore they get accumulated in human organs, causing long term negative health effects. For this reason, maximum contaminant levels have been defined for drinking water by different organizations from all over the world. The current states of strategies for trace heavy metal detection are mainly focusing on chemical and optical techniques. Although these techniques demonstrate excellent sensitivity, their widespread usage is limited by some common drawbacks such as bulky and sophisticated equipment, time-consuming and labor-intensive procedures. These disadvantages make the techniques limited to laboratory applications only.

In this chapter, aiming to explore new heavy metal sensing technology which could be portable and fast responsive. A fundamentally new type of electrical-based LOC sensor is developed based on the coupling of solid-water contact electrification and electrostatic induction phenomena. As shown in **Figure 6.1**, the sensing part consists of a surface functionalized PDMS layer with a gold electrode buried underneath. The PDMS layer functions as a triboelectric material and when water flows

through the microfluidic channel, contact electrification, or triboelectrification occurs between the water molecules and the PDMS. This forms a simplified single-electrode TENG model. When water is pumped into the microfluidic channel and withdrawn back periodically, electron flows are induced in the attached gold electrode, leading to electrical signals like a typical TENG. The surface of the PDMS layer is functionalized with chelating agents for recognition of heavy metal ions. Therefore, the magnitude of the generated signals varies with the heavy metal concentration. By choosing different chelating agents, the as-developed sensor can selectively detect common heavy metal ions, such as Pb^{2+} , with a detection limit of 0.01 mg/L (0.01ppm), which meets the guidelines for heavy metals in drinking water recommended by the World Health Organization (WHO).

Figure 6.1 Image and structural illustration of the microfluidic heavy metal sensor.

6.2 Design and fabrication the microfluidic sensor

As sketch in Figure 6.1, the main body of the sensor is a microfluidic channel binding on a glass substrate. But the microfluidic channel is not directly bonded to the glass. There is one PDMS layer in between, functioning as the triboelectric layer. Underneath the PDMS layer, there is a gold electrode to measure the induced current when water is flowing through the electrode. The surface of the PDMS layer is functionalized with a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), for recognizing heavy metal ions.

The fabrication of the microfluidic sensor is divided into two parts. One is to prepare the glass substrate with a gold electrode, the other is to fabricate the microfluidic channel. Then the two parts can be bonded together using oxygen plasma bonding. The microfluidic channel is fabricated using soft lithography method. A mold is fabricated using SU-8 photoresist by photolithography. Then liquid PDMS is poured onto the SU-8 mold. After curing, the PDMS microfluidic channel can be peeled off from the mold. For the second part, the 2 mm width gold electrode is deposited on a glass slide using E-Beam evaporation. The patterning of the electrode is done by photolithography using AZ5214 as the photoresist. After electrode deposition, an ultra-thin layer of liquid PDMS is spin-coated (2000 rpm) onto the glass slide to cover the entire surface including the gold electrode. Then the PDMS is cured by heating. Since the surfaces are both PDMS, the two parts can be easily bonded together to form a seal microfluidic channel. The surface treatment on the PDMS layer is described in Chapter 6.4.

6.3 Working principle of the triboelectrification-enabled sensor when there is no surface treatment on PDMS layer

The working mechanism of the sensor is the same as the single-electrode TENG described in Chapter 2.1. The contact electrification causes positive and negative charges to accumulate on the surfaces of flowing water and the PDMS layer respectively. During measurement, water is periodically pumped and withdrawn in the microfluidic channel. As illustrated in **Figure 6.2a**. At the beginning, since the PDMS surface is negatively charged, the local electric field will induce electron flow from the gold electrode to earth, resulting in positive charge on electrode. When water is approaching the gold electrode, the local electric field on the PDMS is neutralized by water. Thus, electrons flow from the earth back to the electrode to maintain an overall neutral environment. When water is withdrawn, the PDMS surface becomes negatively charged again so that electrons are induced to flow from the electrode to earth. Therefore, whenever water is pumping through or withdrawn back through the gold electrode, a triboelectric current is generated and the two currents are opposite in direction. This current signal can serve as a parameter for detecting heavy metal ions. The magnitude of this current signal is dependent on the flow velocity and surface charge on the PDMS layer. This phenomenon enables the self-powered operation of the sensor, which requires no additional power source but only an electrometer to acquire the measuring signals. **Figure 6.2b** is the currents generated when DI water is pumped in/withdrawn out periodically in a sensor that has not undergone surface treatment. It needs to highlight that the sensor without surface treatment cannot be used for heavy metal detection yet because the PDMS surface is not functionalized for metal recognition and capture.

Figure 6.2 Working principle of the triboelectrification-enabled heavy metal sensor. (a) The illustration of electron flows when water is passing and withdrawn through the gold electrode. [126] (b) The induced current signals when water is periodically pumped and withdrawn in the microfluidic channel.

6.4 Procedures for Surface Treatment

The goal of surface treatment is to introduce chelating agents on the PDMS surface. Chelating agents, such as EDTA, are chemical compounds that have high affinity to metal ions. They tend to form multiple coordinate bonds with metal ions to form a stable, water-soluble complex. Thus, metal ions are retained on the PDMS surface, causing change in surface charge. As a result, the generated triboelectric current is changed.

The surface functionalization of the PDMS is through silanization reaction [127]. Briefly, the as-fabricated chip is first undergoing oxygen plasma treatment to generate hydroxyl (-OH) groups temporarily on the PDMS surface. After that, 3-Aminopropyl-triethoxysilane (APTES) was diluted with pure ethanol in a ratio of 1:3 and the solution was flowed into the microfluidic channel for 5 mins. Then the microfluidic channel was then washed with ethanol two times followed by introducing 30% ammonium hydroxide solution into the channel for 3 mins. Then the channel was washed with DI water three times. The advantage of silanization reaction is that reactive amine (-NH_2) groups are introduced on the PDMS surface so that the surface now is open to accommodate various molecules containing -COOH groups, such as ethylenediaminetetraacetic acid (EDTA) and glutathione. The procedures for surface treatment are illustrated in **Figure 6.3a**. The successful coating of EDTA can be verified by looking at the triboelectric current generated. As shown in **Figure 6.3b**, there is a tremendous drop in the current signal after surface treatment. This is because that the PDMS surface is now covered by EDTA molecules so that the surface cannot accommodate negative charges.

(a) Procedures for Surface Treatment

Figure 6.3 (a) Procedures for surface functionalization with chelating agents, EDTA. (b) Change in current signals before and after surface functionalization with EDTA.

6.5 Detection of heavy metals

In the process of heavy metal detection, a small amount of testing solution (<50 μl) was first injected into the microfluidic sensor and wait for 1 min, followed by rinsing with DI water. This step is to allow heavy metals to be captured by the chelating agents. Then DI water is pumped in/withdrawn back periodically with a syringe pump at a flow rate of 100 $\mu\text{l}/\text{min}$ to measure the current signals. **Figure 6.4a and 6.4b** show the current signals obtained from EDTA-coated microfluidic sensors for Pb^{2+} solutions of different concentrations. It is observed that the 0.1 ppm solution is able to generate very distinguishable results (above 25 pA) as compared to DI water (less than 2 pA). The magnitude of the current signals increases as the Pb^{2+} concentration goes up. This might be attributed to the increased metal ions captured by the chelating agents, resulting in an increased surface charge.

Since the current signals are also dependent on the flow velocity of water, charge transfer might be a better parameter in calibration of the heavy metal concentration because charge transfer is velocity independent. The charge transfer is calculated as the integration of the curve of current versus time. **Figure 6.4c** is the calibration of the measured charge transfer with Pb^{2+} concentration from 0.01 to 100 ppm.

Figure 6.4 Lead ions detection using the functionalized microfluidic sensor. (a) and (b) The current signals in response to DI water, 0.1 ppm, 1ppm and 10ppm Pb^{2+} solution. (c) Calibration of the measured charge transfer with Pb^{2+} concentration.

6.6 Selective detection of Pb^{2+}

Selectivity is a critical feature for a sensor. If a sensor is highly selective for detecting Pb^{2+} , that means this sensor would not be less responsive to other metals. The triboelectrification-enabled microfluidic sensor could be highly selective in detecting particular heavy metals if the sensing surface is functionalized with chelating agents that have a strong affinity to the metal of target [128]. In order to demonstrate selective detection of Pb^{2+} , glutathione is adopted as the surface chelating agent as it exhibits a relatively strong affinity to Pb^{2+} . A group of metals at the same molar concentration ($1\ \mu\text{M}$) were measured using the glutathione coated sensor. The charge transfers measured are normalized as the ratio to the charge transfer in Pb^{2+} detection and plotted in **Figure 6.5**. It can be observed that the charge transfer with Pb^{2+} sample was much larger than that for other metals, indicating a good selectivity towards Pb^{2+} .

Figure 6.5 Test of the selectivity of the triboelectrification-enabled microfluidic sensor for Pb^{2+} detection by using the glutathione as the surface modifying agent.

6.7 Conclusion

In the chapter, a triboelectrification-enabled microfluidic heavy metal sensor was developed. In the detection of Pb^{2+} ions, 0.01 ppm detection limit was achieved, which is meeting the standard recommended by the WHO. Selective detection of Pb^{2+} ions was also demonstrated by using glutathione functionalized sensor. More importantly, this sensing method is based on electrical properties so that the detection process is fast and not requiring sophisticated equipment. Hence, this sensor has great potential to be developed into portable devices that can be used out of the laboratory.

Chapter 7: Conclusion

In this thesis, the great potentials of TENG technology and electrical-based LOC devices for biological applications have been discussed and demonstrated. The recent concept of TENG has been utilized for developing a small size, batteryless implantable treatment device. The first TENG-based self-powered IDD was demonstrated with its functionality for ocular drug delivery. Pumping flow rates from 5.3 to 40 $\mu\text{L min}^{-1}$ under different rotating speeds of TENG have been realized. As a proof-of-concept, *ex vivo* trans-sclera drug delivery in porcine eyes was performed using the TENG-based self-powered IDD by harvesting the biokinetic energies of human. This work underlines the innovations in TENG-enabled self-powered IDD for developing novel therapeutic approaches in treating chronic diseases.

TENG was also utilized in nanowire-assisted electro-transfection system for direct gene delivery without using chemical carriers. The system can be simply driven by tapping a contact-separation TENG and no extra energy source equipment was needed. After therapeutic siRNAs were electro-transfected into cancer cells, the K-ras gene expression in MiaPaCa-2 cells was down-regulated by $46 \pm 6.29\%$, and the bcr-abl gene expression was reduced by $31.6 \pm 6.67\%$ in K562 cells. These results are comparable to those when using commercialized chemical transfection agents, such as Lipofectamine 2000. However, using the TENG powered transfection system is much cheaper and more straightforward because the raw siRNA can be delivered directly without any treatment. As compared to the conventional electro-transfection set-up, our TENG powered system can achieve much higher cell viability (90% compared to less than 70% in conventional setup) because the current output from TENG is intrinsically small, which prevent damaging cells due to Joule heating. Overall, a novel smart gene

electroporation device is demonstrated and deserves further exploration in mRNA delivery, pDNA delivery, CAR-T and CRISPR/CAS9 gene editing system.

For electrical-based LOC devices, applications of TEER measurement and heavy metal detection were presented. In general, electrical-based devices may have a narrow range of applications in the biomedical realm, but their advantages are apparent as well. These devices are usually fast in response, non-disruptive to samples and requiring no sophisticated equipment to function.

Author's Publications

- C. Yang, **G. Yang**, Q. Ouyang, S. Kuang, P. Song, Y. Zhang, G. Xu, D.P. Poenar, G. Zhu, K.T. Yong, Z.L. Wang, “Nanowire-Array-based Gene Electro-Transfection System Driven by Human-motion Operated Triboelectric Nanogenerator” , (Status: accepted by *Nano Energy*).
- P. Song, **G. Yang**, T. Lang, K.-T. Yong, “Nanogenerators for wearable bioelectronics and biodevices”, *Journal of Physics D: Applied Physics*, 52 (2019) 023002.
- Q. Ouyang, X. Feng, S. Kuang, N. Panwar, P. Song, C. Yang, **G. Yang**, X. Hemu, G. Zhang, H.S. Yoon, J.P. Tam, B. Liedberg, G. Zhu, K.-T. Yong, Z.L. Wang, “Self-powered, on-demand transdermal drug delivery system driven by triboelectric nanogenerator”, *Nano Energy*, 62 (2019) 610-619.
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- **G. Yang**, R.W.L. Ang, D.P. Poenar, Q. Ramadan, W.K. Wan, “Tissue-on-a-Chip for Extended Viability and Trans-Epithelial Electrical Resistance (TEER) Assessment of Gastrointestinal Tissue Biopsies” , (Status: in preparation).

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