行政院國家科學委員會專題研究計畫　成果報告

心室輔助器在心衰竭動物模型利用微流體通道上離子運動特性的研究及其相關之應用

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-234-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
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計畫主持人：周迺寬
共同主持人：管傑雄

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計畫類別：■個別型計畫　□整合型計畫

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□國際合作研究計畫國外研究報告書一份

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一.中文摘要

心室輔助器對於心臟衰竭病患等待心臟移植前提供比傳統藥物冶療更好的生活品質與一年後存活率，由於病人在心室輔助器支持下期間會發生生理訊號的變化以及神經元的離子運動對神經信號的傳輸的表現探討分析，就顯的十分重要。若能以工程角度探討其物理機制，則對於神經相關疾病的診斷甚至進一步療程的發展都有相當大的助益。對離子運動的物理機制，試著以所知的理論模型來解決。離子的多元性與它們之間複雜的交互作用，生物系統內的離子運動並不易於了解。而為了簡化模擬系統，通常利用建立於矽晶圓上的簡單生物系統，特別是微流道，作為研究離子運動的環境，並進而尋求流體內離子運動的解答。在計畫中，以矽或砷化鎵相關的晶圓處理技術發展微流道模型，透過離子與電子運動的相似性，對固態電子的元件物理的理解，輔助對微流道中離子運動特性的認知。生物系統中離子運動相當重要。為了瞭解神經傳導，研究鈉離子在通道中的物理機制以及其所產生的空間電荷、電場為重要關鍵。這份計畫主要在研究離子的移動特性。透過半導體製成技術，可以製造出含有奈米構造的微流道結構，來模擬神經中的陽離子通道，藉著量測此通道在不同偏壓條件下的電流響應，可以分析離子的移動特性與物理機制。

關鍵詞:心室輔助器、離子運動、

二.英文摘要

Abstract

As the use of ventricular assist device (VAD) has increased dramatically during the past decade, it is necessary to understand the hemodynamic change and interaction between VAD and physiological ionic signal transmission. The improvement for Taita No.1 VAD was still persist no matter the preparation of polyurethane, endothelial cell culture prevent thrombosis, newer streamline of impeller design, the biocontroller development that matches physiological requirement, and spectrum analysis of flow and pressure. All things have to make additional efforts. Because physiological signal changes will happen when patients are under VAD-treatment, ion movement versus expressions of neural signal transmissions becomes extremely important. By selecting a point of view in engineering to discuss physical mechanisms occurred in ion movement that is much helpful in clinical diagnosis and the following therapeutic development. We introduce the basic knowledge of a neuron in the following sections to illustrate that why we are interest in physical mechanisms in ion movements and we also try to solve the problem according to some models as far as we know. Though the signal transmitted of neuron of different types has sizable differences, the form of signals are basically the same, all consist of change of the electric potential on the cell membrane of neuron. The electric potential perturbation produced by a part of cells will be spread to the surrounding area gradually, and then finished the information

transmission. Unless the signal amplification caused of energy supplement acquired by neuron, with the increase with the distance among the signal sources and neuron, the perturbation will gradually weaken.

Keywords: **Ventricle assist device, Ion channel**

Introduction

Recently a remodeled smaller T-LVAD for canine model was established for acute phase study. Since the first domestic case of heart transplantation had been carried out in the department of cardiac surgery of National Taiwan University Hospital (NTUH) in 1987, more than one hundred cases of experiences for various surgical treatments of the end-stage myocardiac diseases had been accumulated so far. We also applied some mechanical-assisted circulating systems enthusiastically that make patients suffered from severe end-stage cardiomyopathy and unable to wait for the suitable heart transplant at a time. Such as ECMO implantated subcutaneously, composed of centrifugal pumps and membrane/porous fiber oxygenator, formed an extracorporeal cardiopulmonary circulation which lightens cardiac load, increases tissue oxygen saturation level, and remain regular functions of other important organs like brain, kidney, and liver, etc. But there were general unhealthy inflammations have been commonly observed after using ECMO 5 to 7 days, it means that ECMO cannot be used for a long time. Besides, the huge volume of the machine is still troublesome. So NTUH imported the first Thoratec VAD from abroad to extend the circulation assist of patients who are waiting for an organ transplant. The structural principle of Thoratec VAD is a 65 cc extracorporeal blood housing driven by a pneumatic diaphragm, which makes patients cumbersome. In our cases, four patients who accepted heart transplant after using Thoratec VAD for 8, 48, 55, and 298 days, but the volume is still large because of the air compressor requirement in the driving device. It means that patients are still inconvenient in motion. In addition, HeartMate VAD clinically applied on 7 patients in NTUH, four of them who accepted heart transplant after using Thoratec VAD for 287, 222, 67, and 46 days. Because its 83cc blood chamber is imbedded in the patient's abdomen, which is unsuitable for the smaller physique of Asian human, and its also the pneumatic type diaphragm, though its driving device is relatively light, the volume is still very large and difficult to take along. Owing to the cost and maintenance charges of VAD systems used in America and Europe is expensive, and the blood chamber is always too large that make it unsuitable for the body surface less than $1.5m^2$/Kg of Asian women. Base on the biotechnology is our important industry in the 21st century so NTUH began to develop impeller centrifugal pump ventricular assist devices. Its unique streamline and idea of superconductor-driving device had been admitted by domestic and abroad artificial organ academic organizations and, received only 0.02 beats with blood flow of hemolysis index *in vitro*. In the experiments of calves survives in initial stage and ten mini pig's acute tests, we did some measurement like routine, biochemical and hemolysis tests to demonstrate that the lethal hemolysis could not occur when patients using Taita No.1 VAD, or the hemolysis is lower than animal's RBC regeneration ability in the allowed range. In long-time survive of animals, eight calves survived more than 30 days under VAD-treatment. The Taita No.1 VAD development group had passed a patent of the product from National Science Council in Taiwan in1998 (No.87 111 87), and also approved by Bureau of Standards, Metrology & Inspection of R.O.C (No.154105, the period of validity is 16, Dec, 2010). Taita No.1 VAD was still persist no matter the preparation of polyurethane, endothelial cell culture prevent thrombosis, newer streamline of impeller design, the biocontroller development that matches physiological

requirement, and spectrum analysis of flow and pressure. All things have to make additional efforts. Because physiological signal changes will happen when patients are under VAD-treatment, ion movement versus expressions of neural signal transmissions becomes extremely important. By selecting a point of view in engineering to discuss physical mechanisms occurred in ion movement, that is much helpful in clinical diagnosis and the following therapeutic development. We introduce the basic knowledge of a neuron in the following sections to illustrate that why we are interest in physical mechanisms in ion movements and we also try to solve the problem according to some models as far as we know.

Though the signal transmitted of neuron of different types has sizable differences, the form of signals are basically the same, all consist of change of the electric potential on the cell membrane of neuron. The electric potential perturbation produced by a part of cells will be spread to the surrounding area gradually, and then finished the information transmission. Unless the signal amplification caused of energy supplement acquired by neuron, with the increase with the distance among the signal sources and neuron, the perturbation will gradually weaken. The traveling wave of electric stimulus is so-called action potential or neural impulse which can propagate from a neuron end to the other end with 100m/s even quicker speed without attenuation. And the characteristic of the threshold voltage of positive ions is the reason of action potential.

三、材料與方法

Materials and Methods

Healthy mongrel dogs about 12-16 kgs were used for this experiment. After sedated with Chlorpromazine Hydrochloride 25mg (Ying-Yuan, Tainan, Taiwan) for 30 minutes, the dog was anesthetized with an intravenous injection of Thiamylal 0.2 gm (0.5 gm in 20 ml distilled water) (Citosol®—Kyolin, Tokyo, Japan) and maintained with 2-4% of 2-bromo-2-chloro-1, 1, 1-trifluoroethane (Fluothane® — Zeneca, Macclesfield, Cheshire, UK) administrated via an endotrachial tube through which respiration was sustained by a Microprocessor Ventilator 7200 (Puritan-Bennett, Carlsbad, CA, USA). Three electrodes were connected on both forelegs and one hind leg to collect electrocardiograph (ECG). The arterial cannula was inserted into the femoral artery to collect the arterial blood pressure (ABP). A left thoracotomy incision was made in the fourth inter-costal space for the surgical operation.

After pericardiotomy the aortic trunk and pulmonary trunk were exposed, the 12SB Transonic® Flowprobe (Transonic Systems Inc. Ithaca, New York, USA) was encircled on the aorta and the pulmonary artery to measure the cardiac output of the left ventricle and the right ventricle. By clamping the descending aorta, the outlet graft — polytetrafluoroethylene (PTFE) (Gortex®, W.L. Gore and Associates Inc., Flagstaff, AZ, USA) of the pump was sutured onto the sidewall of the descending thoracic aorta with 6-0 prolene (Ethicon, Edinburg, UK).

After exposure of LV apex, the inlet tubing of the pump was inserted into the LV apex and the tip of the tube was faced the mitral valve and ultimately located in the middle of the ventricle to ensure proper blood suction. Then the tube was tied closely with interrupted sutures with 4-0 Prolene (Ethicon, Edinburg, UK) and pledges. After completion of the suture, an incision was made on the xyphoid to pass out the outlet tube of pump, the inlet tube was passed out thru the fourth inter-costal area and both were connected with LVAD pump. "Dacron" was coated on the surface area of the tubes when passed through the skin and sub cutis to make sure that good adhesion could occur. Action potential transmission is really caused by Na^+ movement along the positive ion channel in the space. The principle of action potential transmission could be elucidated by observing Na^+ movement and its distribution

along the axon. In fact, the behavior of Na^+ moving along the axon is quiet similar to the electrons moving along the waveguide! That's why we believe that using engineering model to analyze biosystems is helpful.

According to theoretical model of ion movement described above, we find that electric field and ion concentration are important parameters influencing related mechanisms. We can derive the diffusivity and mobility in different electric field and ion concentration. Current measurement reflects behaviors of ion diffusion and drift:

$$\begin{cases} J_p = pe\mu_p \mathcal{E} - eD_p \dfrac{\partial p}{\partial x} \\ J_n = ne\mu_n \mathcal{E} + eD_n \dfrac{\partial n}{\partial x} \end{cases} \quad (1)$$

Where $J_{p(n)}$ is positive(or negative) current density. Both p and n are time-dependent, so ion movement characteristic can be reflected by current measurement.

By applying different voltages, we measure the corresponding curve of response current to time (I-T).We calculated p and n from Equation (2) and make the comparison of theoretical and experimental values and obtain some important parameters like diffusivity, mobility and conductivity, etc., four oligonucleotides solution as dATP, dTTP, dCTP, and dGTP with each concentration is 100mM, were added in the microfluidic channel. Under 3V applied voltage, we obtain the DC transient response shown in Fig.1.

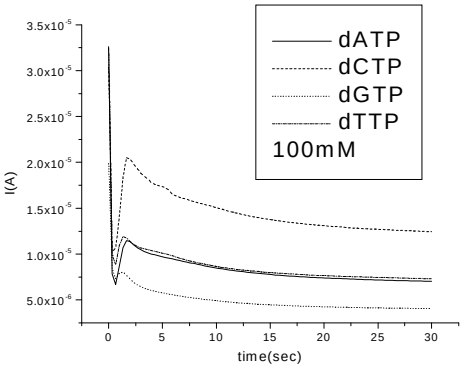


Fig.1 Measurement of I-T curve of dATP,dGTP,dTTP, and dCTP.

There are two special phenomena in the experiment. First is the I-T oscillation between 0 to 3 seconds. Second, it is repeatable that the current value is always the same. The current sequence is: dCTP＞dTTP ＞dATP＞dGTP .

The result discussion and theoretical model analysis derived from Equation (2) are still on processing. By our reasonable prediction, oscillations between 0 to 3 seconds maybe commonly generated by diffusion and drift of the space charges in microchannel. And we also noticed that response current of four DNA reagents are due to their individual molecular weight, suppose it is helpful identifying DNA sequence. We are still make efforts in finding optimal parameters to improve identifying ability. According to our initial result at present, it's wrathful to go on the ion movement research.

The experimental setup for AC transient response is shown in Fig.2. There are both AC and DC component feeded by power supply, while AC component is much lower than DC, so named as small signal. The current output from biochip is amplified by an current amplifier, then the response signals, which contains fundamental frequency and harmonic wave component, can be processed on a signal analyzer.

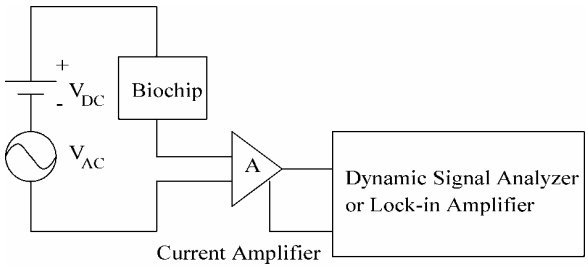


Fig2 A block diagram for the AC measurement system.

The current density we measured is the function of applied bias, which can be represent in Taylor series:

$$J(V_{DC}+V_{AC}\sin\omega t)=J(V_{DC})+\left.\frac{dJ}{dV}\right|_{V_{DC}} V_{AC}\sin\omega t+\left.\frac{1}{2}\frac{dJ}{dV}\right|_{V_{DC}} V_{AC}^2\sin^2\omega t+\Lambda$$

(2)

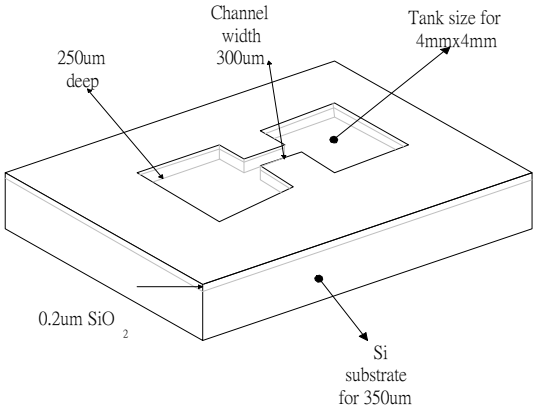
We can calculate dJ/dV and d^2J/dV^2 values by measuring harmonic components. Then we can derive the relationship between differentiation value of current density and ion concentration that in the bottom of the channel. For example, suppose heavy ions (negatively charged) dominate the current tendency, then the value of first derivative of the current density can be rewritten as:

$$\frac{dJ}{dV}=\frac{dJ}{dx}\frac{dx}{dV}=-\frac{1}{\varepsilon_0}\left(\frac{\partial n}{\partial x}e\mu_n\varepsilon_0+eD_n\frac{\partial^2 n}{\partial x^2}\right)$$

. (3)

Where ion concentration in the bottom of the channel is the boundary condition of Equation (2), which is the impartment information in ion movement calculation.

By the way, its noticeable that diversity of various kinds of ions and their complicate interactions among the system, ion movement in the biosystem is still not easy to realize nowadays. In order to simplify the modeling system, we usually use a simple biosystem so named as microfluidic channel environment, that established on the silicon wafer, to take for ion movement research, and find out the solution of ion movement. We will develop the microfluidic channel model by using wafer processing technique related to Si or GaAs in our minor project. We can realize the ion in microfluidic channel via semiconductor physics and devices, then make the comparison of the similarity between ion and electron movement.



Discussion for Ion Movement and Distribution Based on Ambipolar Equation Concepts

We have established a model to illustrate that the impulse measurement is reasonable for observing movement in different charged macroions and getting stable and repeatable signals. To realize ion drift situation in the twinkling of an eye (if the impulse is rapid enough), we only centre on ion drift and diffusion mechanisms instead of discussing other complicated electrochemical mechanisms that happened in longer time scales.

During ion movement under electric field, at t = 0, both positive and negative charged ions are uniformly distributed, diffusion current component is almost not exist, all ions movement produced drift current. As time proceeding, more and more ions with opposite charge property accumulated at the surfaces on the two terminal electrodes, it means that ion concentration difference became evident, diffusion current component increased and drift current component decreased. But there exists a barrier between ions in solution and induced image charges in the metal electrode prevent them combining together. At last, all ions accumulated at two sides and reverse induced internal field cancelled (See Fig.3 (a) to (c)).

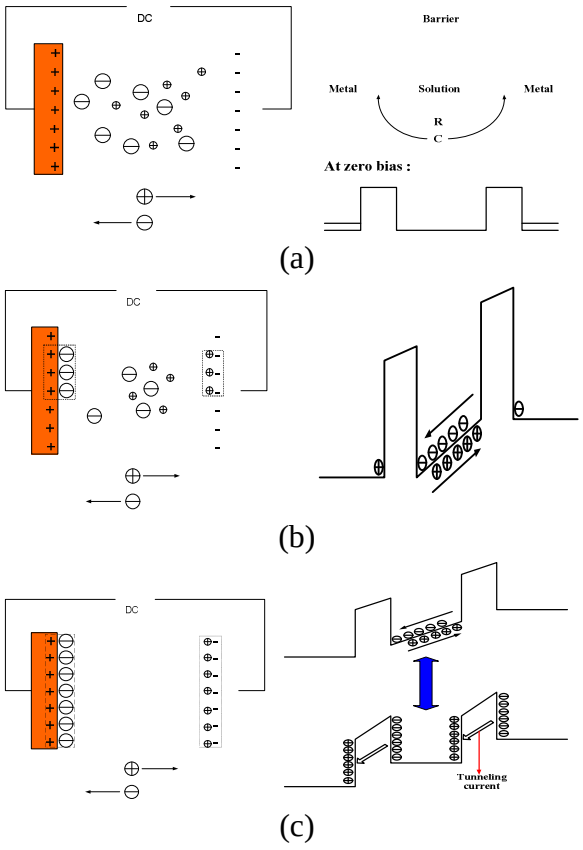


Fig .3 Schematic illustration for the whole procedure during ion movement under applied rapid impulse voltage and its corresponding band diagram evolvement:

(a) Zero bias (t = 0), all drift, no diffusion; (b) As time proceeding, ions with opposite charge accumulated at the two terminal electrodes. Both drift and diffusion are the current component; (c) At theoretical steady-state, all ions adhered to electrodes which induced reversed internal field, there are tunneling current existed in two thin barriers generated by metal-DNA solution interfaces.

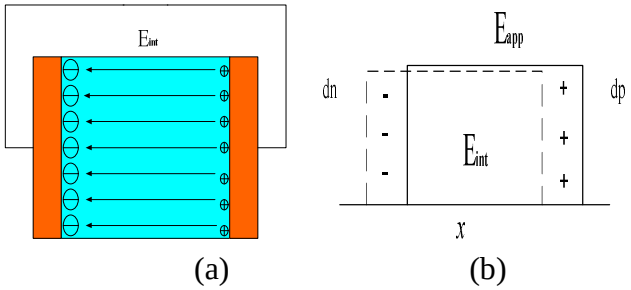


Fig4 (a) At last, all ions accumulated at two sides and reverse induced internal field cancelled applied electric field. (b) Corresponding diagram to (a).

(1) Applied electric field, diffusion current component diminished to zero and drift current component remained increasing again. That's why the peak-like response of I-T curves had been displayed. Note that the rule of charge neutrality should not be disobeyed during the whole procedure, it is the main presupposition.

(2) With regard to damping in the I-T curve, by inference, is due to inertia of macroions because of its slow response. For a groundless talk, when they are affected by reverse field, backward charge field induced inversion current makes peak drop down, but it can't "put on the brakes" immediately, so a transient "back and forth" curve can be observed. To prove our inference, we will try to do the curve fitting by Bessel function in the future.

(3) As we know, the work speed of a semiconductor device always depends on the mobility of minority carriers in a p-n junction of the semiconductor. It means that "Mobility of minority carrier dominates during the procedure of carrier transport". Similarly, like the rate-limiting step of Michaelis-Menton theory (called Bottle-neck mechanism in chemistry reaction), according to Ambipolar equation, heavy ion behavior (DNA) also dominates during our impulse mode observation is also a reasonable prediction. So we can preliminary ensure that the transient response we observed is the behavior of DNA macroions instead of other small cations. We may even establish an Ambipolar equation model for heavy ions (such as DNA) and small ions (such as H^+**.** Na^+) analogized from semiconductor physics.

四、結果與討論

Results

In order to understand the characteristic of ion movement, we will develop two techniques of measurement to achieve the

DC current response and AC harmonic response. We also develop various fabrication processes of biochips at the same time. We decided to research the electric-gas characteristics of biochips based on two technique of measurement. Meanwhile, optical measurement will be coupled in other sub-project for the same chip. Combined analyzing to the electric-gas characteristics and optical properties of ions in solution make us know clearly and thoroughly about the ion movement mechanisms in the microfluidic channel. And we have compared and demonstrated the established theoretical model and our experimental results. At last, we will engage the molecular device production base on ion movement properties.

Biochip preparation on the substrate of GaAs or Si wafer, with one or many gaps, and high depth/width ratio. Preparation skill developed specially deep plasma-etching as a main purpose. To accomplishment of DC measurement system and animal experiment. For measurement and analysis to DC transient response of moving ions in microchannel. To fabricate a biochip with the nanometer-scale gaps on the substrate of GaAs or Si wafer. Then to use computer simulation to current property of ion movement in microchannel of VAD animal model.

Impulse response experimental data exhibition

First, we choose various alternative square waves or impulse with voltages from 18.4 mV to 1.44 V, and frequencies from 100Hz to 1MHz applied on DNA microfluidic chip. By our observation, only impulse period with a short duty cycle makes rapid mechanisms enough to avoid drift and hysteresis, and the other ground period (V_a = **0**) ensures that all DNA samples fallback to the same initial conditions. In the following paragraph, we will discuss our experiment result focusing on positive bias with 5 % duty cycle. Fig 3-4 shows the four DNA transient responses under applying voltage 288 mV with 100 kHz frequency.

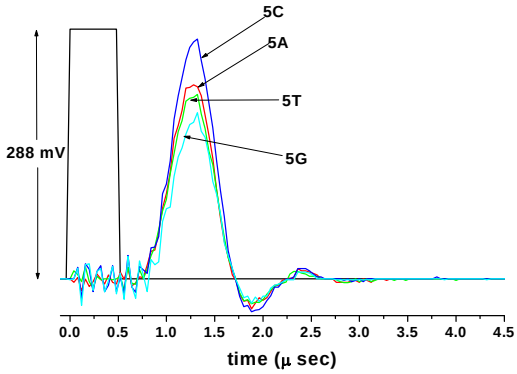


Fig.5 In order to unify initial conditions of all samples able to go back to zero, we decided to reduce duty cycle to 5% and multi-average data, we found that response current of C is always maximum; response current of G is always minimum, but the relationship of A and T are still need to be confirmed (Bias = +288 mV).

	Repeat times	**Peak current ± SD %（μA）**
5A	**30**	**9.2 ± 4.66 %**
5T	**30**	**8.9 ± 5.03 %**
5C	**20**	**11.5 ± 8.83 %**
5G	**20**	**8.0 ± 7.19 %**

Table.1 Measurement results with the condition of 100 kHz, 288 mV, and 5% duty cycle. In order to make samples 5A and 5T more differentiable, more repeat times are adopted than other two samples. We also showed the peak current values ± standard deviation (SD) percentage as a reference and we give a reasonable assumption for error of adding DNA solution sample via the pipet.

In our measurement, there are also some important issues in biomolecular ion movement and distribution under AC electric field：During the procedure of electrophoresis, diffusion current dominate or drift current dominate of DNA movement during applied voltage stimulating process? Whatever, if we can find out that in what time scale, the repeatability of current response curves

decreases obviously, we can make time-scale of electron affect on biomolecules more clear.

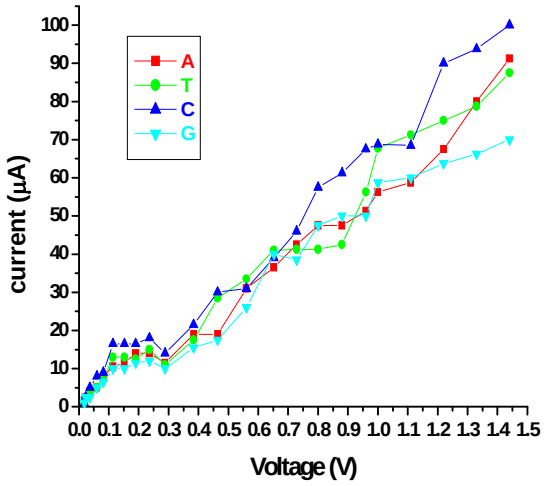


Fig.5 The I-V characteristics of 5A, 5T, 5C and 5G from 18.4 mV to 1.44 V. At lower voltage (< 300 mV) the current order is approximately remain as C>A>T>G without remarkable variation. But at higher voltage (>300 mV), the current order persistent changes.

Ion movement induced backward space charge field, which leads to voltage alternation between R and C, in other words, the resistance and capacitance of DNA seems not a constant.

It seems that RC product equals a constant but individual R and C is not always remain unchanged. Because in switching electric field, voltage alternation is still remaining between R and C, that makes R ↑ C ↓ ,then R ↓ C ↑ to go round and begin again oscillation. At present, microelectrophoresis analysis with a rapid impulse measurement is ideally practicable. We have preliminary acquired some stable and repeatable signals of the novel research, and we also got the approximately I-V characteristics (from 18.4 mV to 1.44 V), but it can still be improved in the future.

Discussion

A microfluidic channel structure can be manufactured which contains nanostructures via semiconductor fabrication process to simulate the positive ion channel in nerve fibers. The characteristic of ion movement and related physical mechanisms are analyzed by measuring current responses in the channel with different bias conditions. We can develop new devices only in molecular dimensions by using the results in the project. In this project, we have brought up a concept base on semiconductor physics to analysis the movement of DNA macroions under an applied impulse voltage. We obtained the DNA composition with different molecular weights rapidly with just feeding an impulse with high-frequency instead of really “separating” them. By observing RC transient response of different bases, molecular weights and concentrations, we expected that the response curve under the same measuring condition can be repeated. Then we also imported Ambipolar equations in semiconductor physics to elucidate DNA movement and distribution under applied impulses. And we got an I-V characteristic after sufficient times for measuring, analyzing current component and the reasons of current difference among different DNA sequences. We preliminary confirmed the novel analysis technique is practicable and repeatable. In order to understand the characteristic of ion movement, we developed two techniques of measurement to achieve the DC current response and AC harmonic response. We also initially developed various fabrication process of biochips at the same time. By researching the electric-gas characteristics of biochips based on two technique of measurement. The optical measurement technique will be integrated in other sub-project for the same chip. Combined analyzing to the electric-gas characteristics and optical properties of ions in solution make us know clearly and thoroughly about the ion movement mechanisms in the microfluidic channel. And we have compared and demonstrated the established theoretical model and our experimental results. At last, we also have

established a preliminary architecture for the molecular device production base on ion movement properties. After sufficient data are being acquired, we decide to establish a theoretical model to analyze biomolecular rapid mechanism, and then making a breakthrough for biomedical diagnosis and identification.

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